STUDIES ON THE MEDICINALLY IMPORTANT RHYNCHOSIA (PHASEOLEAE, FABACEAE) SPECIES: TAXONOMY, ETHNOBOTANY, PHYTOCHEMISTRY AND ANTIMICROBIAL ACTIVITY

BY

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DECLARATION

I **Mashiane Sonnyboy Mothogoane**, declare that this dissertation submitted by me for the degree of Masters in Botany at the Department of Botany and Plant Biotechnology in the Faculty of Science at the University of Johannesburg is my own work in design and execution. It has not been submitted before for any degree or examination at this or any other academic institution and that all the sources that I have used or quoted have been indicated and acknowledged by means of complete references.

15/04/2019

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SIGNATURE DATE

(MR M S MOTHOGOANE)

DEDICATION

I would like to dedicate this work to my family, parents, Sejeng Magdeline Mothogoane and Mmile William (late) for all the encouragement throughout my school years. I am also deeply grateful to wife, Lorraine Phumzile Mothogoane and my children, Dayan Uriah Mothogoane and Lemuela Amalia Mothogoane, for all their love, support and inspiration throughout this study project.

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ABSTRACT

The genus *Rhynchosia* Lour. was first introduced by Loureiro, to accommodate the species *Rhynchosia volubilis* Lour. It derives its name from the Greek word "*rhynchos*" which means a beak, snout or horn, in reference to the shape of the keel petals. *Rhynchosia* is a relatively large genus with more than 230 species occurring throughout the tropics and subtropics, with about 77 species indigenous to southern Africa. Within the subtribe Cajaninae, *Rhynchosia* is closely related to the genus *Eriosema* (DC) Desv. However, it can be distinguished from the latter by the structure of the funicular attachment of the seed in relation to the hilum. In *Rhynchosia* the funicular attachment is in the centre of the hilum, while in *Eriosema* the attachment is at the end of the hilum. It also has affinities with the genus *Bolusafra* Kuntze, however, previous preliminary molecular studies have shown that *Bolusafra* is congeneric with *Rhynchosia*. This project is part of ongoing taxonomic studies in the type section *Rhynchosia*, and its main aim was to revise the *R. minima* complex and provide a synopsis of the ethnomedicinally important species. The secondary aim of the study was to screen these plants for their antimicrobial activities based on their traditional use. However, due to unavailability of some of the plants in the field, despite numerous attempts, not all species could be screened. The third aim was to conduct qualitative evaluation of the phytochemical properties of these plants. The morphological studies were conducted by examining herbarium specimens and during field surveys, while type specimens were studied online. Taxonomic studies revealed that there are five infraspecific taxa in the *R. minima* complex in southern Africa, these include two newly described varieties (*R. minima* (L.) DC. var. *glandularis* M.S. Mothogoane and Moteetee and *R. minima* (L.) DC. var. *magniflora* M.S.

Mothogoane and Moteetee). A taxonomic key to the infraspecific taxa of *R. minima* complex is provided, in addition, the correct nomenclature, typification, and distribution maps for all taxa studied are presented. Various literature sources and databases were consulted for the compilation of ethnomedicinal *Rhynchosia* species. The survey showed that in addition to *R. minima,* there are eight other ethomedicinally important species of *Rhynchosia* species in southern Africa, namely; *R. adenodes* Eckl. & Zeyh., *R. albissima* Gand., *R. caribaea* (Jacq.) DC., *R. densiflora* (Roth) DC., *R. harveyi* Eckl. & Zeyh., *R. hirta* (Schumach.) Meikle, *R. resinosa* (Hochst.ex A.Rich.) Meikle, and *R. sublobata*. These species are used for the treatment of various human infections, including gastrointestinal disorders, respiratory tract infections (RTIs), skin conditions, and sexually transmitted infections (STIs). However, only three of these species (*R. adenodes, R. caribaea* and *R. harveyi*) are used medicinally in southern Africa, while the others are used elsewhere across their wider geographical distribution ranges. Based on the microbial inhibitory concentrations (MIC) results, *Rhynchosia hirta* and *R. sublobata* showed noteworthy antibacterial activities against *Escherichia coli* and *Staphylococcus aureus*, which are the major causative pathogens for gastrointestinal problems such as diarrhoea (This explains the efficacy of these species in the treatment of stomach problems). The studied species (*R.adenodes*, *R.albissima*, *R. caribaea*, *R.hirta*, *R.minima* and *R.sublobata*) were found to contain a number of chemical constituents including, alkaloids, coumarins, flavonoids, phenols, saponins, tannins, and terpenoids, which might explain the ethnobotanical uses of these species in traditional medicine.

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LIST OF ABBREVIATIONS, ACRONYMS AND SYMBOLS

ºC	degree celsius
!	Specimen seen by reseacher
%	percent
†	Specimen does not exist anymore
Aq.	Aqueous
ATCC	American type culture collection
BM	British Museum
BOL	Bolus Herbarium
DCM	Dichloromethanol
DME	Dimethyl Ether
DMSO	Dimethyl sulfoxide
EtOH	Ethanol
g	gram
GRA	Selmar Schonland Herbarium
h	hour
H_2O	water
INT	p-iodonitrotetrazolium iodide
JRAU	University of Johannesburg Herbarium
K	Royal Botanic Gardens Herbarium
L	liter
LC	Least Concern
m.a.s.l.	Meters above sea level
MeOH	Methanol
mg/ml	milligram per milliliter
MIC	Minimum Inhibition Concentration
ml	milliliter
NBG	Compton Herbarium
NH	KwaZulu-Natal Herbarium
NU	Bews Herbarium

Org	organic
PRE	Pretoria National Herbarium
SAM	South African Museum Herbarium
SANBI	South African National Biodiversity Institute
TSB	Tryptone soya broth
µl	microliter
µg/ml	microgram per milliliter
WIND	National Herbarium Namibia

CHAPTER 1: INTRODUCTION

1.1 General Introduction

The genus *Rhynchosia* Lour. is a member of the legume family Fabaceae, subfamily Papilionoideae, tribe Phaseoleae, and subtribe Cajaninae (Lackey, 1981). The Fabaceae is the third largest angiosperm family after the Asteraceae and Orchidaceae in terms of species count. The family has a cosmopolitan distribution, with close to 770 genera and over 19 500 species worldwide (Lewis et al., 2005; LPWG, 2013; 2017). Fabaceae is a morphologically, physiologically, and ecologically diverse plant group, and comprises species which exhibit variable growth habit, ranging from small annual herbs to climbing annuals, or perennials with tendrils, desert shrubs, aquatics, and large tropical trees (LPWG, 2017). The flowers range from radially symmetrical (actinomorphic) to bilaterally symmetrical (zygomorphic), and asymmetrical (LPWG, 2017) and are adapted to pollinators such as insects, birds and mammals. In southern Africa, Fabaceae is the third largest angiosperm plant family with 155 genera and 1 516 species after Aizoaceae and Asteraceae (Koekemoer et al., 2014). Fabaceae represent the most significant ecological constituents that are found in all major biomes of the world and also occur in the most extreme habitats (Schrire, 2005). Legumes are only second to Poaceae in their importance in human utilization (LPWG, 2017). They have been used by man to enrich the soil through their nitrogen fixing abilities (through symbiosis with bacteria-*Rhizobia*) for many centuries (Graham, 1941; LPWG, 2017). According to Graham (1941), records of the oldest civilizations of both Egypt and eastern Asia bear witness to the usefulness

of various legume plants such as beans, peas, vetches, soybeans, and alfalfa. Theophrastus, one of the early Greek botanists is reported to have stated that leguminous plants are “reinvigorating” to the soil (Graham, 1941). They are important food crops that are highly nutritious sources of proteins and micronutrients (Yahara et al., 2013). Grain and forage legumes account for 27% of the world’s primary crop production, with grains alone contributing 33% of protein nitrogen dietary requirement for humans (Cullen, 1997, Graham and Vance, 2003). In the United States of America, legumes are the most important family of honey-producing and erosion control plants (Graham, 1941). Legumes also provide man with other numerous uses such as gums and tannins from *Acacia* species (Graham, 1941). In the recent publication by the LPWG (2017), six monophyletic subfamilies: Caesalpinioideae, Cercidoideae, Detarioideae, Dialioideae, Duparquetioideae, and Papilionoideae are recognised. The schematic diagram showing the phylogenetic diversity of the six Fabaceae subfamilies is illustrated in Figure1.1.

1.1.1Subfamily Papilionoideae

Papilionoideae (Faboideae) is by far the largest subfamily in Fabaceae and can be distinguished by its papilionoid flowers, asymmetrical seeds, ovate-elliptical cotyledons, campylotropous ovules, and curved or a short spiral embryo axis (Lackey, 1981; El-Gazzar et al., 2013). Globally, it comprises ca. 503 genera and ca. 14 000 species, and has a cosmopolitan distribution (LPWG, 2017). There are 29 recognised tribes within this subfamily (Lewis et al., 2005; De Queiroz et al., 2015), as indicated in Table 1.1.

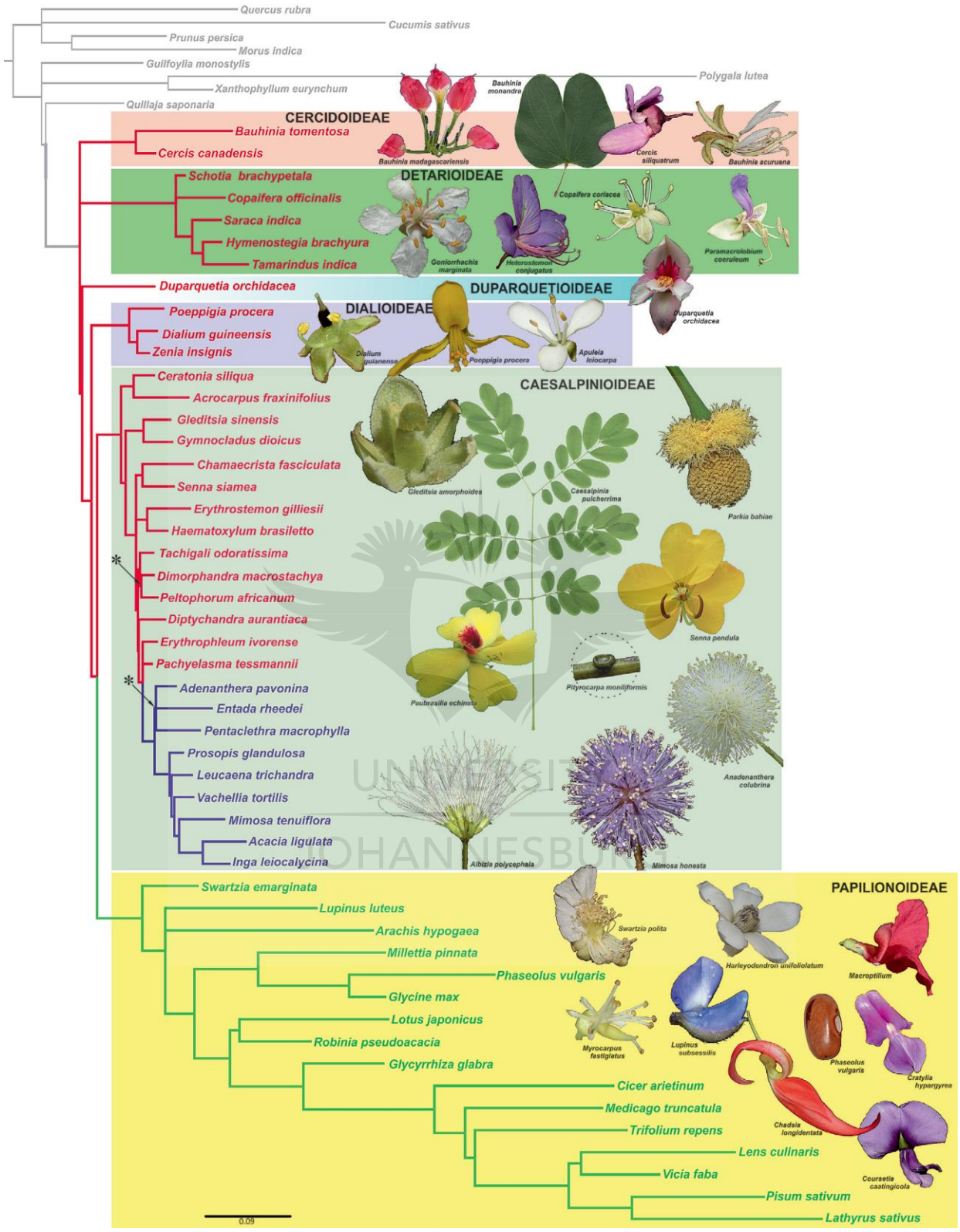

Figure1.1: Phylogenetic and subfamily classification of Fabaceae, based on LPWG (2017).

Table 1.1: Tribes recognised in Papilionoideae, adapted from Lewis et al. (2005)

Tribe	Number of genera	Number of species
1. Abreae (Wright & Arn.ex Endl.) Hutch.	1	17
2. Amorpheae Boriss.	8	245–248
3. Bossiaeeae (Benth.) Hutch.	6	ca.72
4. Brongniartieae (Benth.) Hutch.	10	ca.152
5. Cicereae Alef.	1	43
6. Crotolarieae (Benth.) Hutch.	11	ca.1204
7. Dalbergieae Bronn ex DC.	49	1319–1331
8. Desmodieae (Benth.) Hutch.	30	524–530
9. Diocleae (Benth.) Hutch.	14	ca. 200
10. Dipterygeae Polhill	3	ca. 22
11. Euchresteae (Nakai) H. Ohashi	1	ca.4
12. Fabeae Rchb.	5	ca. 329
13. Galegeae (Bronn) Dumort	24	2880–3180
14. Genisteae (Bronn) Dumort	25	551–572
15. Hedysareae DC.	12	400–453

16. Hypocalypteae (Yakovlev) A.L. Schutte	1	3
17. Indigofereae Benth.	4	ca. 710
18. Loteae DC.	22	ca.282
19. Millettieae Miq.	45	904–914
20. Mirbelieae (Benth.) Polhill & Crisp.	25	686–689
21. Phaseoleae (Bronn) DC.	75	Ca. 1438
22. Podalyrieae Benth.	8	125
23. Psoraleeae Lowe	6	ca. 135
24. Robinieae (Benth.) Hutch.	21	ca. 145
25. Sesbanieae (Rydb.) Hutch.	1	ca.60
26. Sophoreae Spreng. ex DC.	45	393–398
27. Swartzieae DC.	17	ca.258
28. Thermopsideae Yakovlev	6	43–46
29. Trifolieae (Bronn) Endl.	6	ca.485

1.1.2 Tribe Phaseoleae

With over 80 genera and 1 500 species, Phaseoleae is the largest tribe in the subfamily Papilionoideae, and arguably the most economically important in the family (Lackey, 1981). It has a worldwide distribution, but occurs predominantly in the tropics, with the majority of the species found in Africa and the Americas. Most of the species in this tribe are important bean plants, used by all cultures in many ways (Mackinder et al., 2001). Within the family, the tribe is closely related to the tribes Millettieae, Abreae, Psoraleeae and Desmodieae (Wojciechowski, 2003). An earlier account by Lackey (1981) recognised eight subtribes: Cajaninae, Clitoriinae, Diocleinae, Erythrininae, Glycininae, Kennediinae, Ophrestiinae and Phaseolinae. However, based on recent molecular analyses of the tribe, only six subtribes are currently recognised (Cajaninae, Clitoriinae, Glycininae, Kennediinae, Ophrestiinae and Phaseolinae); Erythrininae has been disintegrated and excluded from the original subtribes (Lewis et al., 2005), while Diocleininae was elevated to tribal status (De Queiroz et al., 2015). The tribe is characterised by the presence of multicellular glandular hairs throughout, but especially large and prevalent in the Kennediinae and Phaseolinae (Lackey, 1981).

1.1.3 Subtribe Cajaninae

Cajaninae is characterised by the presence of vesicular glands and bulbous-based hairs. The vesicular glands are characterised by a squat head of cells contained within a shallow depression of the epidermis. These glands occur on the calyces, ovaries, stems, lower surfaces of leaves and fruits (Lackey, 1981, Moteetee and Van Wyk, 2006). The subtribe is characterised by trifoliolate leaves (sometimes unifoliolate), and the absence of

bracteoles, with the exception of *Adenodolichos* Harms (Lackey, 1981; Moteetee and Van Wyk, 2006). Subtribe Cajaninae comprises eight genera, namely; *Adenodolichos* Harms, *Bolusafra* Kuntze, *Cajanus* DC., *Dunbaria* Wright & Arn., *Eriosema* DC. ex Desv., *Flemingia* Roxb*. ex* Ait., *Paracalyx* Ali. and *Rhynchosia* Lour. and a total of ca. 495 species with a wide distribution in paleotropics to warm temperate regions of the old world and the neotropics and subtropics in the new world (Verdcourt, 2001; Lewis et al., 2005). The subtribe includes the economically important species *Cajanus cajan* (L.) Millsp. (Van der Maesen, 2003). In southern Africa, only five genera occur naturally, i.e. *Bolusafra* Kuntze, *Cajanus* DC., *Eriosema* (DC.) G. Don, *Flemingia* Roxb. ex W.T. Aiton and *Rhynchosia* Lour. (Germishuizen, 2000), although preliminary molecular data indicates that *Bolusafra* is part of *Rhynchosia* (Manyelo, 2014).

1.2 Taxonomic History of *Rhynchosia*

The genus *Rhynchosia* Lour. was first established by Loureiro in 1790 to accommodate the species *Rhynchosia volubilis* Lour. (Hutchinson, 1964). The name *Rhynchosia* is derived from the Greek word "*rhynchos*" which means a beak, snout or horn, in reference to the keel petals (Clarke and Charters, 2016). Within the subtribe Cajaninae, *Rhynchosia* is closely related to *Eriosema* (Fortunato, 2000, Schrire, 2005). *Rhynchosia* can, however, be distinguished from *Eriosema* in the structure of the funicular attachment of the seed in relation to the hilum (Grear and Dengler, 1976). In *Rhynchosia* the funicular attachment is in the centre of the hilum, whereas in *Eriosema* the attachment is at the end of the hilum (Lackey, 1981). It also has affinities with the genus *Bolusafra*, however, despite the preliminary molecular data (Manyelo, 2014) which shows that *Bolusafra* is embedded within *Rhynchosia*, it differs from *Bolusafra* in that *Rhynchosia* seed arils are

obsolete or almost absent in most of the South African species, with compressed and often falcate fruits as contrary to the prominent seed arils and turgid fruits of *Bolusafra* (Verdcourt, 2001; Moteetee and Van Wyk, 2006). In her revision of *Rhynchosia* taxa occurring in the United States of America, Vail (1899), recognised 16 taxa, however, she used the genus name *Dolicholus* Medikus and recorded *Rhynchosia* as a synonym. For the next 60 years after this publication, the names *Dolicholus* and *Rhynchosia* were both used in literature, often interchangeably until 1959, when the name *Rhynchosia* was conserved against *Dolicholus* (Woods and Key, 2009). *Rhynchosia* is a relatively large genus of more than 230 species occurring throughout the tropics and subtropics, including the warm regions of the world (Verdcourt, 2001, Lewis et al., 2005). With the advancement of botanical research in the 20th century, more species have been added to the *Rhynchosia* species list since the first species was described, through the work of several botanists. These include De Candolle (1825) with 51 species worldwide, Ecklon and Zeyher (1836) with 12 African species, Harvey (1862), who recognised 29 species occurring in the region known then as the Cape Colony (now separated into the Eastern Cape, the Northern Cape and Western Cape Provinces) and Port Natal (Durban), Baker (1929) with 93 species of Tropical Africa, Verdcourt (2001) with 43 species.

The South African species of the genus *Rhynchosia* were last comprehensively revised by Baker (1923), who recognised 59 species and grouped them into five sections namely: *Cyanospermum* Benth., *Arcyphyllum* Torr. and Gray, *Chrysoscias* Benth., *Polytropia* Harvey and Sonder, and *Rhynchosia* Lour. (*EuRhynchosia*). However, the molecular phylogenetic study by Manyelo (2014) concluded that the sectional classification of Baker (1923) needs to be re-investigated and sections clearly defined for the groups in the type

section. Sections *Cyanospermum, Polytropia* and *Chrysoscias* have been revised (Moteetee et al., 2012, 2014; Jaca et al., 2018, respectively), while a recent study (Jaca and Moteetee, 2018), indicated that section *Arcyphyllum* is not represented in southern Africa. This project is part of ongoing taxonomic studies in the type section *Rhynchosia*.

1.3 Ethnobotany

Tribal people of the world, especially in the continents of Asia and Africa have been utilizing natural products from plants for their health and food since times immemorial. The African traditional medicine hugely relies on plants which tribal communities have been using for centuries (Chhabra and Mahunnah, 1994; Hutchings et al., 1996; Neuwinger, 2000). Southern Africa is richly blessed with a great diversity of plants that account for about 30 000 species of flowering plants; these species constitute 10% of the whole world's flora (Van Wyk and Smith, 2001). According to Van Wyk and Gericke (2018), the rural people of this region use a wide variety of plants in their daily lives for various uses such as sustenance, water, medicine, building houses, and fuel. However, ethnobotany in this region is still underdeveloped discipline, and more work still needs to be done, in so far as the documentation of indigenous knowledge uses of plants is concerned, before it is lost to current and future generations (Van Wyk and Gericke, 2018). It is estimated that in the southern African region alone, about 4 000 species are used as medicine (Van Wyk and Gericke, 2018). In South Africa, most rural people, are dependent on indigenous plants for food and medicine; people in these areas are hugely affected by high unemployment levels, insufficient government health services, and also continued traditional belief systems (Huntley, 1995). The ethnobotanical importance of members of the family Fabaceae is unequivocal. This is exemplified by the fact that

numerous studies across the globe have repeatedly shown that Fabaceae is either the most dominant family or ranks among the most popular families in their ethnobotanical use, mostly as food and medicine, such as *Cajanus cajan* (L.) Millsp., *Cassia fistula* L., *Cassia senna* L., *Lablab purpureus* (L.) Sweet, *Pisum sativum* L., *Sphenostylis stenocarpa* (A. Rich.) Harms, *Vigna radiata* (L.) R. Wilczek, *Vigna unguiculata* (L.) Walp., etc. (Galaso et al., 1995; Kritzinger et al., 2005; Saeed and Tariq, 2005; Khattoon and Ali, 2006; Loganayaki et al., 2011; Upadhyay et al., 2011; Hossain et al., 2012; Daniel and Celestina, 2013; Singh et al., 2014; Tang et al., 2014). For example, the legume plant *Pisum sativum* L., is a major vegetable that occurs in all temperate zone countries and its seeds are rich in phenolic antioxidants, which contribute to antimicrobial activity against *Helicobacter pylori* and is also used for the treatment of acne (Saeed and Tariq, 2005). This study explores our current knowledge on the ethnomedicinal uses of *Rhynchosia*, which is dealt with in Chapter 4.

1.4 Phytochemistry

Traditional medicine is a valuable source in the discovery of pharmaceutical drugs in general. Plant products such as natural antioxidants maybe of great help in preventing the progress of various diseases associated with oxidative stresses (Chaturvedi, 2015). Phytochemistry is the study of chemical compounds derived from plants. These compounds can be primary or secondary metabolites found in different parts of the plant, including roots, stems, leaves, flowers, fruits and/or seeds, and are useful in the development of new synthetic drugs (Chew et al., 2011; Saxena et al., 2013). According to Saxena et al. (2013), approximately 4 000 chemical compounds have been listed and classified according to their protective function, physical, and chemical characteristics.

However, of these, only 150 phytochemicals have been studied in detail. It is believed that these compounds are used by plants as defence mechanisms against insects and herbivores, as well as signal compounds to attract pollinators and contribute to colour, aroma, and flavour (Saxena et al., 2013; Wink, 2013). Due to its economic importance, the phytochemistry of the family Fabaceae has been intensively studied. For example, although outdated, Bisby et al. (1994), listed over 4 000 chemical constituents in 2 000 species of the family. In recent years, several phytochemical compounds have been described in Fabaceae (Wink, 2003). Studies on a number of genera in the Fabaceae, for example *Acacia* Mill., *Bauhinia* L., *Cajanus* DC., *Canavalia* DC., *Cassia* L., *Caesalpinia* L., *Calliandra* Benth., *Lablab* Adans., *Leucaena* Benth., *Mucuna* Adans., *Peltophorum* (Vogel) Benth., *Phaseolus* L., *Piliostigma* Hochst., *Sphenostylis* E. Mey., *Vicia* L., etc. have shown that legumes are a source numerous phytochemical constituents that can be grouped into several categories: alkaloids, phenolics, steroids, terpenes, saponins, etc. (Jimoh and Oladiji, 2005; Chew et al., 2011; Loganayaki et al., 2011; Nyananyo and Nyingifa, 2011; Marimuthu and Gurumoorthi, 2013; Parvin et al., 2013; Pasricha et al., 2014). The study on wild and common south Indian legumes by Marimuthu and Gurumoorthi (2013), found the presence of several phytochemicals including alkaloids, steroids, triterpenoids, flavonoids, tannins, phenols, glycosides and saponins in both the wild and common legume species (*Mucuna pruriens* (L.) DC., *Macrotyloma uniflorum* (Lam.) Verdc., *Phaseolus lunatus* L. and *Canavalia ensiformis* (L.) DC.). Tang et al. (2014), listed 56 chemical compounds identified from the mung bean and its sprouts (*Vigna radiata* (L.) Wilczek).

Phytochemical compounds, such as alkaloids and amines, peptides, phenolics, and terpenoids have antimicrobial properties (Wink 2013). Studies on the chemistry and biological activities of these chemical compounds, show that an individual compound or combination of compounds might be of great use in the promotion of good health and eradication of diseases. For example, flavonoids possess many biochemical properties and has excellent medical efficacy, including its antioxidant, anticancer, anti-inflammatory, hepatoprotective, antibacterial and antiviral activities (Kumar and Pandey, 2013). The phytochemistry of *Rhynchosia* is further discussed in detail in Chapter 4.

1.5. Antimicrobial activity

Antimicrobial agents prevent growth of microorganisms. Plants synthesize a variety of chemical compounds, such as alkaloids and amines, peptides, phenolics and terpenoids that are reported to have antimicrobial activity (Wink 2013). In the last few decades, antibiotic and multid-rug resistance by pathogenic bacteria has been of great concern and presents a huge challenge in the treatment of diseases (Maróti et al., 2011). Multiple-drug resistance is mainly caused by indiscriminate use of commercial antimicrobial drugs employed in the treatment of infectious diseases (Albayrak et al., 2012). It warrants an urgent action to develop new drugs and novel antibiotics that can be used effectively to eradicate pathogens.

The antimicrobial activity of chemical compounds and extracts from species of the family Fabaceae has been intensively studied in recent years. For example, lectins and protease inhibitors, as well as tannins isolated from the seeds of *Canavalia ensiformis* (L.) DC. are reported to have antimicrobial activity against several microbial pathogens, i.e. *Bacillus*

subtilis, *Escherichia coli*, *Klebsiella pneumoniae, Proteus vulgaris*, *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Streptococcus pyrigens* (Pugalenthi et al., 2010). In a study by Parvin et al. (2013), the leaf extracts of *Lablab purpureus* (L.) Sweet was found to possess good antimicrobial properties against gram positive bacteria (*Bacillus cereus*, *B. megaterium*, *B. sarcina lutea* and *S. aureus*), gram negative bacteria (*E. coli*, *P. aeruginosa*, *Salmonella paratyphi*, *S. typhi*, *Shigella boydii*, *S. dysenteriae*, *Vibrio mimicus* and *V. parahemolyticus*) and fungi (*Candida albicans*, *Aspergillus niger* and *Saccharomyces cerevacae*), with a zone of inhibition ranging from 8 to 14 mm. The root extract of the rosary pea (*Abrus precatorius* L.), a common plant in India, was found to possess good antibacterial activity against *S. aureus* infection, and thus provides hope and potential as a baseline information for the treatment of skin diseases and diarrhoea (Mistry et al., 2010). The fresh seeds of the velvet bean (*Mucuna pruriens* (L.) DC., demonstrated good antibacterial activity when tested against *Escherichia coli* and *Staphylococcus aureus* (Borhade, 2017). According to Chew et al. (2011), the aerial parts (flowers and leaves) of *Caesalpinia pulcherrima* (L.) Sw. demonstrated a strong antibacterial activity against *Bacillus cereus* and *Shigella dysenteriae*, while the leaves of *Peltophorum pterocarpum* (DC.) K. Heyne displayed a strong antifungal activity (>50 % inhibition) against *Fusarium sp*., *Aspergillus sp*. and *Cladosporium cucumerinum*. A study by Hossain et al. (2012) found that the leaves of *Cassia senna* L., widely found in most tropical regions, have good antibacterial activity against both gram-positive (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative strains (*Escherichia coli* and *Vibrio mimicus*). The antimicrobial activity results of selected *Rhynchosia* species are reported in Chapter 4.

Aims and objectives of the study

- To conduct taxonomic studies of *Rhynchosia* species used traditionally for medicinal purposes, with an emphasis on the *Rhynchosia minima* complex within type section *Rhynchosia*, but excluding the *Rhynchosia totta* complex (already revised by Moteetee and Le Roux (2016)).
- To evaluate their ethnobotanical uses, phytochemical properties as well as antimicrobial activity of the medicinally important *Rhynchosia* species in southern Africa.

Specific objectives:

- Conduct a taxonomic revision of the *Rhynchosia minima* complex
- Identify and record medicinally important *Rhynchosia* species
- Provide synopses of these species, resolve the taxonomic and nomenclatural uncertainties within species, and record their geographical distributions
- Assess the plants for their phytochemical properties
- Screen the unstudied medicinally important *Rhynchosia* species from southern Africa for their antimicrobial activity, based on ethnobotanical uses.

CHAPTER 2: A TAXONOMIC REVISION OF THE *RHYNCHOSIA MINIMA* COMPLEX

2.1. Introduction

Rhynchosia minima (L.) DC. was first described by Linnaeus (1753) as *Dolichos minimus* L, a species with trifoliolate, rhomboid leaves, racemous inflorescences and compressed fruits. De Candolle (1825) transferred the species to the genus *Rhynchosia* DC, wherein he provided a more detailed description. The species was further described by Harvey (1862), Perkins (1907), Baker (1923), Bailey (1930), Verdcourt (1971), Poston (1980), Verdcourt (2001), as well as Du Puy and Labat (2002). It was described as a twining herb, with several slender stems growing from a woody rootstock, broadly ovate-rhomboidal to orbicular, glabrescent to densely pubescent, and glandular leaves, small (6-9 mm) yellow flowers with brown or mauve stripes, borne on axillary racemes, and oblong-falcate, glabrescent to densely pubescent (sometimes with long priestly hairs) pods. *Rhynchosia minima* is commonly known as least snout bean (Jia et al, 2015) and is widely distributed around the world. It occurs in almost all tropical and subtropical countries in Asia, Africa, Australia and the Americas (Hemsley, 1888; Vail, 1899; Verdcourt, 2001; Diamond, 2015). In his treatment of *Rhynchosia* in Flora Tropical East Africa, Verdcourt (1971) divided *R. minima* into seven varieties based mainly on habit, leaf shape, indumentum, flower size, fruit size and shape. However, only four of these varieties (*R*. *minima* (L.) DC. var. *falcata* (E.Mey.) Verdc., *R*. *minima* (L.) DC. var. *memnonia* (Delile) Meikle, *R*. *minima* (L.) DC. var. *minima* and *R*. *minima* (L.) DC. var. *prostrata* (Harv.) Meikle) occur in southern Africa. Verdcourt (1971), when working on Flora Tropical East Africa, identified three species complexes within the section *Rhynchosia* remarking that “three groups

stand out in the genus for their difficulty, so far as Africa is concerned". The three groups in question are *Rhynchosia minima* (L.) DC. complex, *Rhynchosia viscosa* (Roth.) DC. complex and *Rhynchosia totta* (Thunb.) DC complex. However, among the three groups, according to Vercourt (1971), "the worst is unquestionably the *R. minima* group", with regard to taxonomic complexity in the genus. The *R. viscosa* group does not occur in southern Africa, while the *R. totta* group has been revised by Moteetee and Le Roux (2016). The southern African contingency of the *R. minima* complex has never been studied in much detail, therefore, this study presents a more detailed revision of the *R. minima* complex.

2.2 Materials and Methods

Morphological data was gathered from literature, field surveys in Pretoria and surrounding areas, Gauteng and Mpumalanga Provinces during summer, and herbarium specimens housed at SANBI herbaria (PRE, NBG, and NH) and those that were loaned from BOL, GRA, NU and WIND (abbreviations according to Holmgren et al., 1990). More than 300 specimens were examined using a WILD M3Z dissecting microscope and Zeiss Stereo microscope to study and measure the leaves, indumentum, stipules, petiole, flower, inflorescence and fruits. Plant height, growth form, and habitat information was obtained from herbarium specimen labels. All measurements were made in millimetres. Flowers were softened using water and a window cleaning liquid (Trade name: *Windowlene*-an alkaline solution of surfactants and solvents containing 1 to 3% each of 3-butoxy-2-propanol and 1-methoxy-2-propanol). The flowers were soaked overnight and then dissected using a WILD M3Z microscope, mounted in glycerol, and photographed under the microscope (Nikon SMZ 745T) using Samsung J7 cell phone camera, then hand

drawn. Dissection of flowers was performed to study the following characters: the shape and the dimensions of the calyx lobes, the shape and the dimensions of the petals, as well as the shape and the vestiture of the ovary. For each character, five measurements were done and then averaged. The floral and inflorescence characters were measured: length of peduncles, flower size, calyx lobes and tubes, width of upper, lateral and carinal calyx lobes, length and width of standard, wing and keel petals. Fruit measurements were made from herbarium specimen material for each taxon. The flowering and fruiting periods were recorded from the herbarium specimens. Geographical distribution data of each taxon was obtained from specimen labels. The co-ordinates (longitude and latitude) were recorded based on Leistner and Morris (1976) and maps at the Pretoria National Herbarium (PRE). Maps for each species were compiled using Arc GIS Map 1.4 program. The type specimens were studied online (https://plants.jstor.org).

2.3 Taxonomic treatment

2.3.1***. Rhynchosia minima*** (L.) DC., Prodr. 2: 385 (1825). *Dolichos minimus* L., Sp. Pl.: 726 (1753); Baker in F.T.A. 2: 219 (1871) *pro parte*; Baker f., Legum. Trop. Africa: 471 (1929); Hauman in F.C.B. 6: 166 (1954); Meikle in Kew Bull. 9: 275 (1954); in Mem. New York Bot. Gard. 8: 421 (1954); in F.W.T.A., ed. 2, 1: 555 (1958); Torre in C.F.A. 3: 311 (1966); Verdcourt in Kew Bull. 25:101 (1971); in F.T.E.A., Leguminosae, Pap.: 756 (1971); Drummond in Kirkia 8: 226 (1972); Jacobsen in Kirkia 9: 161 (1973); Lock, Leg. Afr. Checklist: 432 (1989). *Dolicholus minimus* (L.) Medikus, in Vorles. Churpf. Phys. Ges. 2: 354 (1787). Type: Jamaica, Herb. Sloane 3.79; typotype: BM. Illustration in Sloane, Voy. Jamaica 1: t.115, f.3 (1707). [Lectotype designated by Vercourt, in Turland & C.E. Jarvis, Taxon 46:469 (1997)].

Perennial, climbing or twinning (sometimes trailing) herbs, stems glabrous to velvety, slender, rootstock woody. Leaves alternate, trifoliolate, (7)9–67×8–55 mm, green (bluish-green, yellowish-green) and grey or silver, rhomboid, ovate or suborbicular, lateral leaves oblique; acuminate, acute or rounded at the apex, cuneate, rounded or truncate at the base, glabrescent to velvety, densely covered with golden glands beneath (or sides in *R. minima* var. *glandularis*); petiole 35–80 mm long; rachis 2–12 mm long; petiolules 0.5–1.5 mm long; stipules small, 1–6×0.5–1.0 mm, linear-lanceolate to . Inflorescences axillary racemes, usually rather lax; rachis 20–180 (250) mm long; peduncle 10–180 mm long. Flowers small, 3–12 mm long; pedicels 1.0–1.5 mm long; bracts minute, deciduous, 2.0×0.5 mm. Calyx pubescent, tube 1–5 mm long; lobes 2–7 mm long, triangular to lanceolate, the lower most the longest, the upper pair joined from ± half their length to almost their entire length. Standard petal yellow, sometimes with dark red veins or flushed red, 4–10×3–7 mm, obovate, puberulous or pubescent, glandular. Wing petals yellow, oblong or obovate, shorter than the keel. Keel petals greenish-yellow, tinged brown, oblong, apically rounded, claw linear. Ovary oblong, 1.5–4 mm long, densely or slightly pubescent, often covered with glands; Style 2–7 mm long, curved upwards and glabrous; Stamen tube 5–8 mm long, with 10 stamens, nine fused to the stamen sheath, vexillary stamen free, only attached at the base of the stamen sheat. Fruits 6–22(25)×3–6(7) mm, oblong-falcate or semi falcate, laterally compressed, glabrescent or sparsely to densely pubescent, and often with long hairs and golden glands, pale olive-green when mature, split open on the upper margin and becoming boat-shaped, 1 or 2 seeded. Seeds brown, grey or blackish when mature, often speckled, 2.5–3.2×2.0–2.5×1.2 mm, oblong-reniform.

Key to the ***Rhynchosia minima*** complex:

1a Leaflets distinctly acute, glabrescent or sparsely pubescent; petiole, 10–80 mm long; flowers small, 3–8 mm long……..2

1b Leaflets obtuse, pubescent to velvety; petiole, 10–60 mm long; flowers large, 4–12 mm long……..3

2a Glands on the lower leaf surface only; petiole short, 10–60 mm long…. ***R. minima*** **var. *minima***

2b Glands on lower and upper surfaces of the leaf; petiole 20–80 mm long……..***R. minima*** var. ***glandularis*** var. ***nov.***

3a Petiole long, 10–60 mm long; flowers 4–10 mm long……..4

3b Petiole short, 10–40 mm long; flowers 9–12 mm long……..***R. minima*** var. ***magniflora*** *var*. ***nov*.**

4a Stems thinly pubescent and silvery……....***R. minima*** var. ***prostrata***

4b Stem densely pubescent to velvety and grey……..….***R. minima*** var. ***memnonia***

2.3.1.1. ***Rhynchosia minima*** (L.) DC. var. ***glandularis*** M.S. Mothogoane and Moteetee, ***var.*** **nov.** Type: Namibia, 2217 (Windhoek), Munisipale Gebied Windhoek, Rooi kwarshounde grond (-CA), 28 November 1962, *Hanekom, W.J. 329* (PRE, holo.!, WIND!, isotype).

Perennial prostrate herb with long trailing stems. *Leaves* yellowish-green, terminal leaflets 7–35×11–40 mm, glabrescent or pubescent; glands prominent on both sides of the leaf surface (Figure 2.1G); petiole length 20–80 mm long; stipules 2–4×0.5–1 mm,

linear to narrowly ovate. *Inflorescences* axillary racemes, 40–150 mm long. *Flowers* small, 5–8 mm long. *Calyx* tube 4 mm long, upper lobes 3–4 mm long, lower lobes 4 mm long, lateral lobes 2–3.5 mm long (Figure 2.1A). *Standard* 5–7×5(4) mm (Figure 2.1B), wings 3–5×2 (Figure 2.1C), keel 4–5×2(3) mm (Figure 2.1D); ovary 2–4 mm long, covered with yellow glands (Figure 2.1F); style 3–5 mm long (Figure 2.1F); stamen tube 6–7 mm long (Figure 2.1E). *Fruits* 16–17× 4–5 mm, oblong, brown, covered with short hairs. Flowering time: from late spring through to the middle of autumn (November–March).

Diagnostic characters: Leaflets yellowish-green, covered with golden glands on both surfaces, a long petiole, 20–80 mm. This variety is similar to *R. minima* var. *minima,* but can be distinguished by the distribution of glands on the leaves (on both upper and lower surfaces in *R. minima* var. *glandularis* vs on the lower surface only in *R. minima* var. *minima*) and the long petioles (20–80 mm long, compared 10–60 mm long). The epithet refers to the abundance of glands on all parts of the plant, especially the distribution which is on both sides of the leaf surfaces, whereas other varieties have glands only on the lower surface.

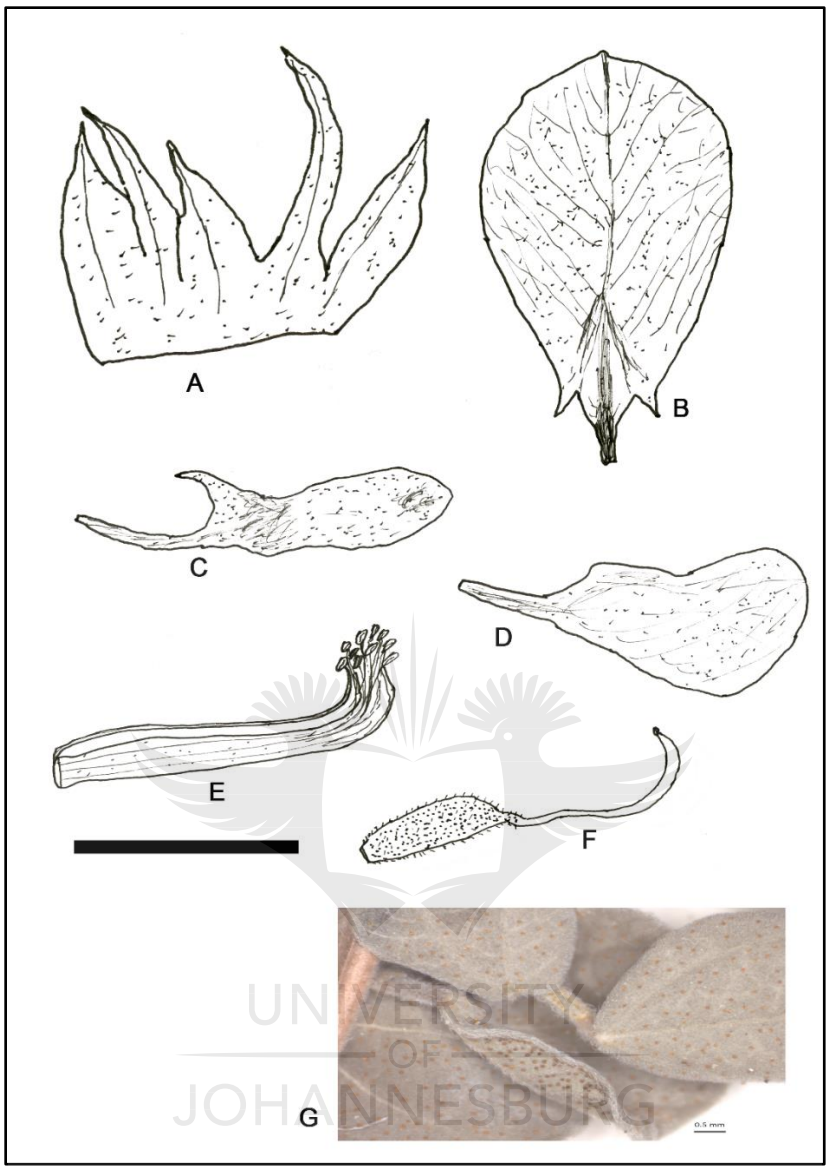

Figure *2.1:* Floral morphology of *Rhynchosia minima* var. *glandularis*: A, calyx opened out with upper lobes to the left; B, standard petal; C, wing petal; D, keel petals; E, stamen sheath, showing nine stamens fused to stamen sheath and the vexillary stamen free; F, Gynoecium (ovary and style). G, Leaflets showing glands distribution and indumentum of *R. minima* var. *glandularis*. Voucher specimen: *Germishuizen 9659* (PRE). Scale bars: A–F = 5 mm, G = 0.5 mm

Distribution and h**abitat:** *R. minima* var. *glandularis* is restricted to Namibia where it grows in grasslands (Figure 2.2). It prefers red sandy soils, among rocks and roadsides.

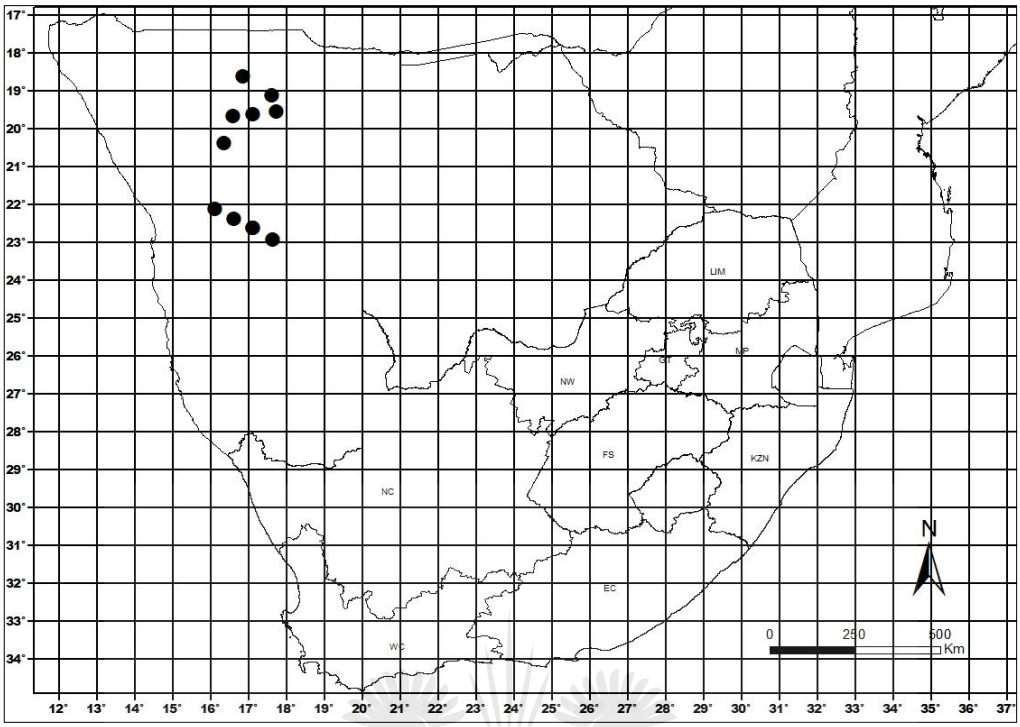

Figure 2.2*:* Known geographic distribution of *Rhynchosia minima* var*. glandularis* in southern Africa

Additional specimens examined

NAMIBIA: **1816** (Namutoni): 17 km *vanaf* [from] *Tsumeb op pad na* [on road to] Otavi. (-DB), 9 February 1974, *Grobbelaar 1896* (PRE). **1916** (Gobaub): 87 km from Otavi on road to Outjo (-DA), 11 March 1997, *Germishuizen 9659* (PRE). **1917** (Tsumeb): Tsumeb (-BA), (Precise date not provided) December 1935, *Boss TRV 35603* (PRE); Tsumeb (-CA), 07 March 1973, *Giess 12474* (PRE); Just north of Kombat on farm Gauss (-DA), 2 March 1995, *Germishuizen 7323* (PRE; WIND). **2016** (Otjiwarongo): Omatjene (-AD), (Precise date not provided), *Liebenberg 4843* (PRE). **2216** (Otjimbingwe): Farm Otjozondu, *auf Höhlenberg vom Seitental aus*, in Felsspalten (-AA), 20 January 1967, *Seydel 4476* (PRE; WIND); 60 km west of Windhoek, on farm Glynberg (-BC), 01 March

1983, *Germishuizen 2471* (PRE). **2217** (Windhoek): Windhoek (-CA), 28 November 1962, *Hanekom 329* (PRE; WIND); 24 March 1964, *Seydel 3917* (PRE; WIND); 5 km north of Dordabis, on tar road to Windhoek (-DC), 28 February 1997, *Germishuizen 9173* (PRE).

2.3.1.2. ***Rhynchosia minima*** (L.) DC. var. ***magniflora*****.** M.S. Mothogoane and Moteetee, ***var. nov***. Type: South Africa, Mpumalanga, 2531 (Komatipoort), Kruger National Park, 17 miles (27.35 km) N.E. of Pretorius Kop, on road to Skukuza (-AB), 23 February 1949, *Codd 5202* (PRE, holo.!)

A perennial twining, prostrate herb, rarely erect. Stems much branched from the base. *Leaves* grey-green, terminal leaflets 10–30(35)×8–28 mm, pubescent; glands on the lower surface of the leaf; petiole length, 10–40 mm long; stipules 2–4(5)×0.5–1 mm, linear to narrowly ovate. *Inflorescences* 30–150 (250) mm long. *Flowers* large (9–12 mm) long. *Calyx* tube 4–5 mm long, upper lobes 5–6 mm long, lower lobes 6–7 mm long, lateral lobes 5–6 mm long (Figure 2.3A). *Standard* 7–10×5(6) mm (Figure 2.3B), wings 4–7×2 (3), (Figure 2.3C), keel 5–7×4 mm (Figure 2.3D); ovary 3–4 mm long, covered with yellow glands (Figure 2.3F); style 5–8 mm long (Figure 2.3F); stamen tube 8–12 mm long (Figure 2.3E). *Fruits* 10–15(20) ×3–5(7) mm, oblong-obovate, brown, covered with short hairs. Flowering time: from early spring through to late autumn (September–April).

Diagnostic characters: *Rhynchosia minima*. var. *magniflora* has grey-green leaves, covered with glands on the lower surface of the leaf. It has large flowers measuring,

9–12 mm long, with a calyx tube of 4–5 mm long, obovate-triangular and the middle vein prominently dark brown (Figure 2.3B). It can also be distinguished by the shape of the

keel, which is almost right-angled (falcate, see Figure 2.3D) and a long style, right-angled at the tip pointing upwards (style 5–8 mm long) (Figure 2.3F). Its fruits are brown, oblong and densely pubescent. The specific epithet '*magniflora'* is in reference to its distinctly big flowers (9–12 mm) as compared to other varieties.

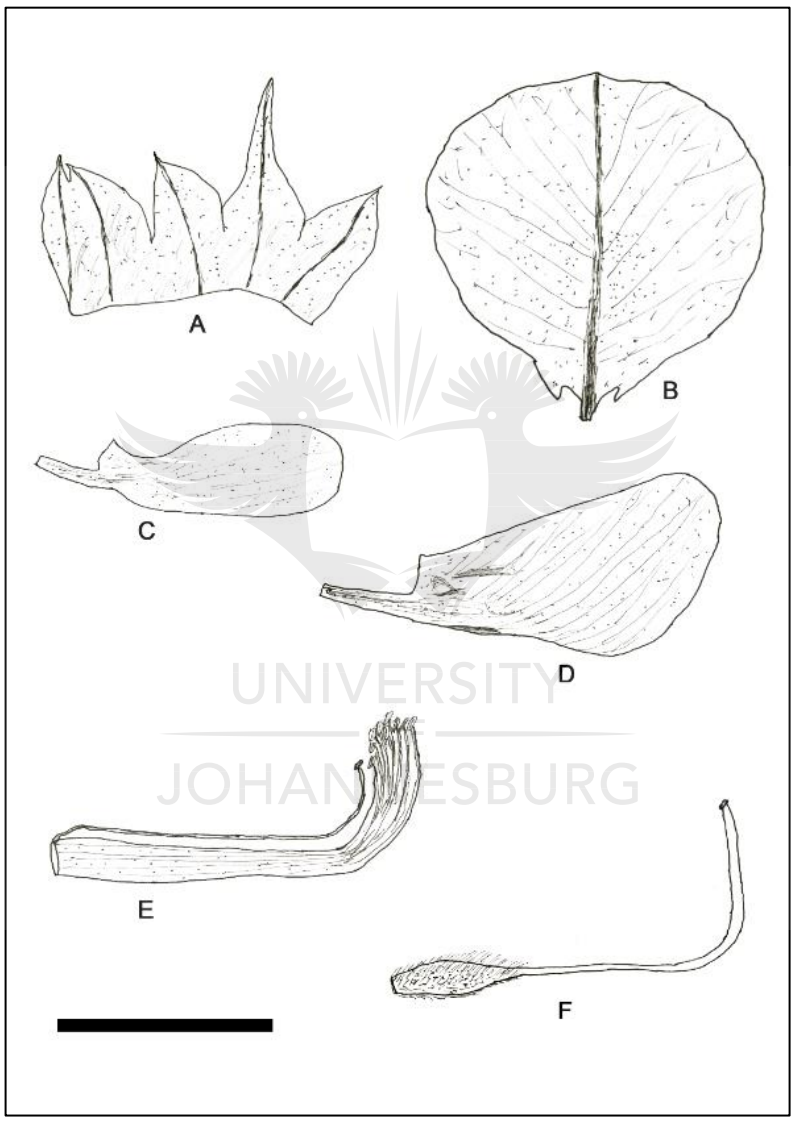

Figure 2.3: Floral morphology of *Rhynchosia minima* var. *magniflora*: A, calyx opened out with upper lobes to the left; B, standard petal; C, wing petal; D, keel petal; E, stamen sheath, showing nine stamens fused to stamen sheath and the vexillary stamen free; F Gynoecium (ovary and style). Voucher specimen: *Crooked 11* (NU). Scale bar: A–F = 5 mm

Distribution and habitat: *Rhynchosia minima* var. *magniflora* is restricted to South Africa (Limpopo, Gauteng, Mpumalanga and KwaZulu-Natal Provinces) and eSwatini in grasslands (Figure 2.4).

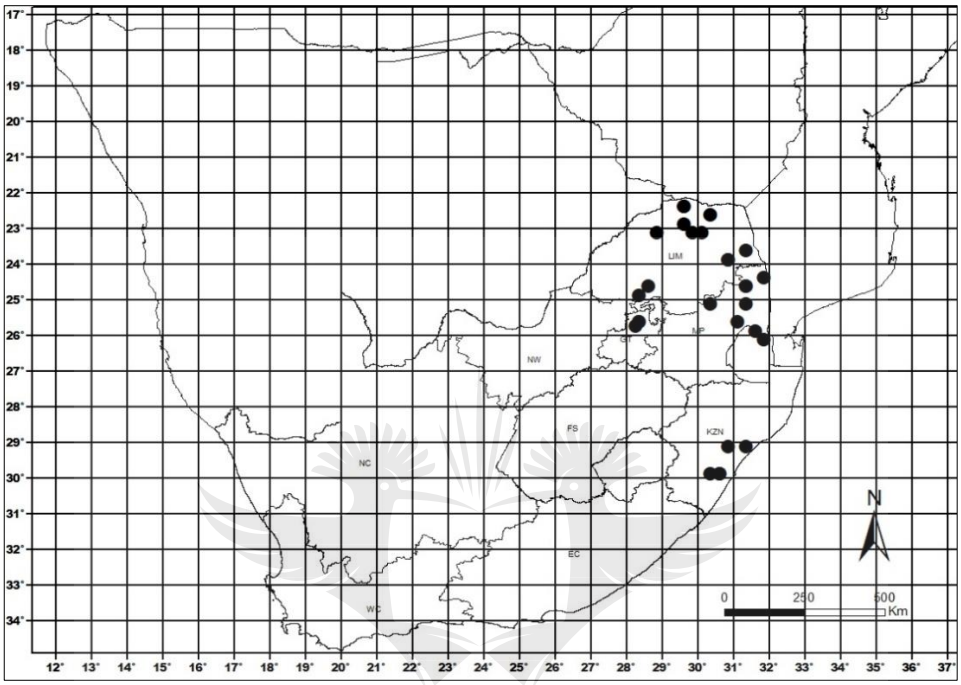

Figure 2.4: Known geographic distribution of *Rhynchosia minima* var. *magniflor*a in southern Africa

Additional specimens examined

SOUTH AFRICA, LIMPOPO PROVINCE: **2229** (Waterpoort): Dongola Reserve (-BC), 21 December 1938, *Smuts and Gillet 4028* (PRE); Dongola Reserve, Dongola Camp, Erfrust, 4.5 miles [7.24 km] NW of camp (-BC), 17 March 1948, *Codd and Dyer 3910* (PRE); Waterpoort (-DC), September 1918, *Rogers 21543* (PRE). **2230** (Musina): Nwanedzi Nature Reserve, Nuanedzi Dam (-CB), 31 January 1980, *Van Wyk 3397* (PRE). **2329** (Polokwane): Blaauwberg (-BB), 10 March 1894, *Schlechter 4647* (PRE);

Louis Trichard (-BB), (Precise date not provided) January 1920, *Breijer TRV 20898* (PRE). **2330** (Tzaneen): Ben Lavin Nature Reserve (-AA), January 1979, *Ben Lavin Grp 58* (PRE); Haenertsburg (-DD), November 1913, *Pott 4637* (PRE); Haenertzburg, hills near Haenertzburg (-DD), 20 October 1938, *Acocks and Hafström 716* (PRE). **2331** (Phalaborwa): Kruger National Park, Sclobe Rand (-CB), 04 November 1952, *Van der Schijf 1178* (PRE). **2428** (Modimolle): Modimolle, Towoomba Exp. Station (-CD), 21 January 1948, *Sidey 1415* (PRE); Mookgophong, Mosdene, Amsterdam Farm (-DA), 17 March 1923, *Galpin M586* (PRE). **GAUTENG PROVINCE**: **2528** (Pretoria): Pretoria National Botanical Garden, north eastern corner of large piece of natural grassland (-CB), 30 November 2006, *Bester 7319* (PRE); 23 October 1980, *Balsinhas 3475* (PRE); (Precise date not provided) 1974, *Dryfhout 764* (PRE). **MPUMALANGA PROVINCE**: **2431** (Acornhoek): Lebombos, Kruger National Park, *helling aan voet van* [slope at the foot of] Lebombos (-BD), 16 March 1976, *Nel 5528* (PRE); Manyeleti Game Reserve, Hermitage (-CB), 05 March 1977, *Bredenkamp 1740* (PRE); 31 December 1991, *Fabian 1416* (PRE). **2530** (Mashishing): 12 km from Mashishing on road to Dullstroom (-AB), 05 March 1979, *Germishuizen 1076* (PRE). **2531** (Komatipoort): Kruger National Park, 17 miles [27.35 km] NE of Pretorius Kop on road to Skukuza (-AB), 23 February 1949, *Codd 5202* (PRE); Barberton phase 2, Mundt's Concession (-CA), 13 February 1998, *Williamson 670* (PRE). **KWAZULU-NATAL PROVINCE**: **2930** (Pietermaritzburg): 20 km to Kranskop from Greytown (-BB), 13 September 1974, *Stirton 1049* (NH); 20 km from Richmond to Pietermaritzburg (-CD), 07 January 1981, *Schrire 453* (NH); Near Eston (-DC), 10 November 1980, *Schrire 456* (NH; PRE). **2931** (Stanger): 42, 7 km from Stanger towards Mtunzini (-AB), 08 December 1973, *Stirton 405* (PRE).

ESWATINI: **2631** (Mbabane): Hlane Game Reserve (-BB), 19 December 1972, *Stephen 1447* (PRE); Tshaneni (-DC), 22 October 1969, *Barrett 72* (PRE).

2.3.1.3. ***Rhynchosia minima*** (L.) DC. var. ***memnonia*** (Del.) Meikle in Kew Bull. 9: 274 (1954) & in Fl. W. Trop. Afr., ed. 2, 1: 555 (1958). *Dolichos memnonia* Del., Descr. Egypte, Hist. Nat. 254, t 38 (1813); Fl. Egypte, Expl. Planches: 110 (1813). *Rhynchosia memnonia* (Del.) DC., Prodr. 2:386 (1825); J.G. Baker in Fl. Trop. Afr. 2:220 (1871); Boiss, Fl. Orient. 2:625 (1872); Baker in Fl. Brit. India, 2: 224 (1879). Type: Delile, Flore d'Egypte, Expl. Planches: t. 38, f. 3 (1813), lectotype, here designated. [Note: The plate shows the trailing growth habit typical of the variety and velvet grey-hairs]

Perennial prostrate (much branched) herb. *Leaves* terminal leaflets 14–36×17–35 mm, obtuse, grey, and densely pubescent to velvety; glands on the lower surface of the leaf; petiole length, 20–35 mm long; stipules 2–6×1 mm, lanceolate, linear to narrowly ovate. *Inflorescences* 30–130 mm long. *Flowers* large, 4–7 mm long. *Calyx* tube 3–4 mm long, upper lobes 4–5 mm long, lower lobes 5–6 mm long, lateral lobes 3–5 mm long (Figure 2. 5A). *Standard* 5–7×5 mm (Figure 2.5B), wings 4(5) ×1.5 mm (Figure 2.5C), keel 4–5×2.5 mm (Figure 2.5D); ovary 2–4 mm long, covered with white hairs and few yellow glands (Figure 2.5F); style 5–7 mm long (Figure 2.5F); stamen tube 6–8 mm long (Figure 2.5E). *Fruits* up to 10–22×4(3)–6 mm, brown, covered with short hairs. Flowering time: from late spring through to late autumn (November–April).

Diagnostic characters: *R. minima var. memnonia* closely resembles *R. minima* var. *prostrata*, with its leaves silky-silvery to velvet or cinereous pubescent on both surfaces, but it can be distinguished by the bigger leaflets sizes (14–36×17–35) as compared to

smaller leaflets in *R. minima var. prostrata (*10–34×10–35 mm). It also has a shorter petiole (20–35 mm) as compared to longer petiole (10–60 mm) of *R. minima* var. *prostrata*.

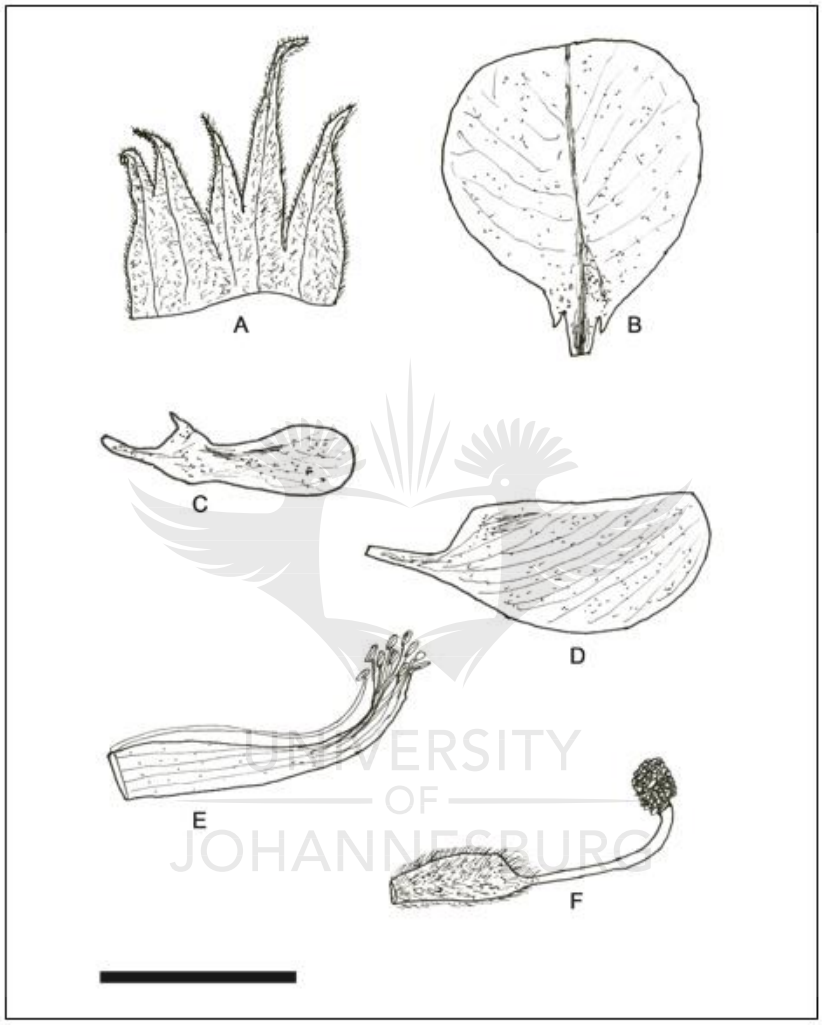

Figure 2.5: Floral morphology of *Rhynchosia minima* var. *memnonia*: A, calyx opened out with upper lobes to the left; B, standard petal; C, wing petal; D, keel petal; E, stamen sheath, showing nine stamens fused to stamen sheath and the vexillary stamen free; F, Gynoecium (ovary and style, with pollen on the stigma). Voucher specimen: *Germishuizen 5141* (PRE). Scale bar: A–F = 5 mm

Distribution and habitat: Within southern Africa, *Rhynchosia minima* var. *memnonia* occurs in Namibia and South Africa (Limpopo, North West, Mpumalanga and KwaZulu-Natal Provinces) in grassland, open mopane woodland and grassy valleys (Figure 2.6). However, specimen records of *R. minima var. memnonia* have been seen from Egypt, Saudi Arabia, West Africa, S. Sahara, Sudan Republic and Ethiopia (Verdcourt, 1971).

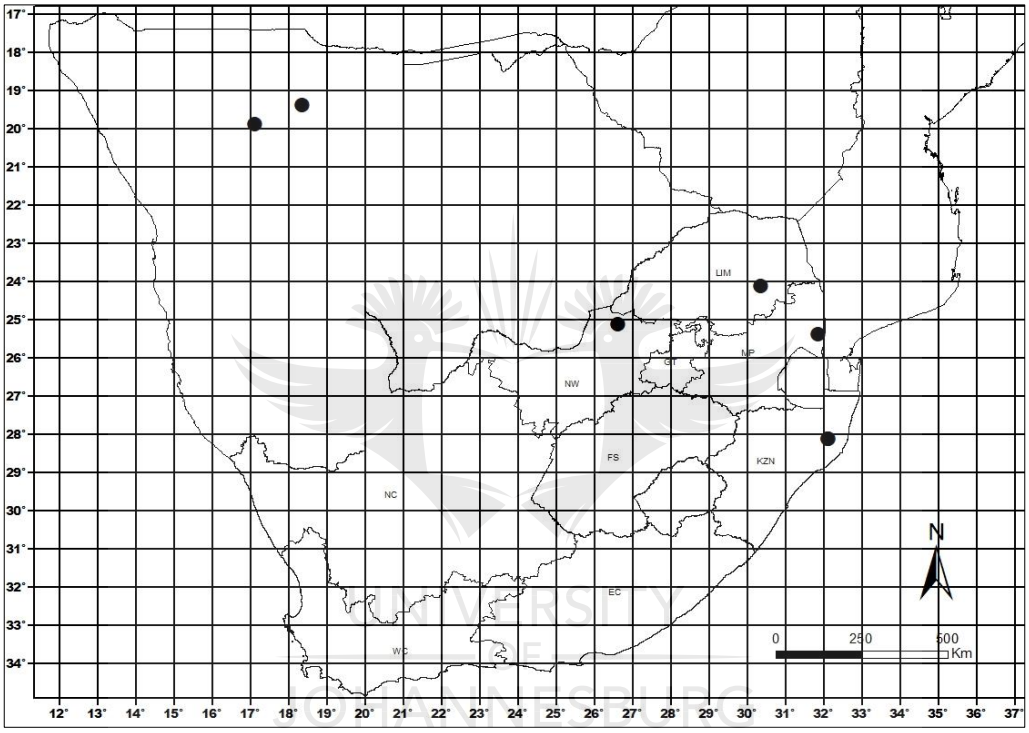

Figure 2.6: Known geographic distribution of *Rhynchosia minima* var. *memnonia* in southern Africa

Additional specimens examined

NAMIBIA: 1917 (Tsumeb): 19, 4 miles [31 km] West of Otavi on road to Otjiwarongo (-CC), 19 March 1955, *De Winter 2844* (PRE). **1918** (Grootfontein): Malta GR 726 Farm (-AD), 24 April 1963, *Giess, Vlok and Bleissner 8431* (PRE).

SOUTH AFRICA, LIMPOPO PROVINCE: 2430 (Pilgrim's Rest), Shiluvane (-AB), 11 November, year not provided, *Junod 768* (PRE). **NORTH WEST PROVINCE**: **2526** (Zeerust): Silkaatskop, ca. 15 km SE along road to Matlapeng Hills (-BA), 09 March 1976, *Van der Meulen 974* (PRE). **MPUMALANGA PROVINCE**: **2531** (Komatipoort), 18 km from Hectorspruit on road to Komatipoort (-BD), 11 March 1989, *Germishuizen 5141* (PRE). **KWAZULU-NATAL PROVINCE**: **2832** (Mtubatuba), Hluhluwe Game Reserve (-AA), 09 April 1954, *Ward 2295* (NU; PRE); Hluhluwe Game Reserve (-AA), 26 November 1955, *Ward 2816* (NU; PRE).

2.3.1.4. ***R. minima*** (L.) DC. var. ***minima***; Meikle in Kew Bull. 9: 275 (1954), in F.W.T.A., ed.2, 1:555 (1958), Torre in C.F.A. 3: 311 (1966), Verdcourt in Kew Bull. 25: 103 (1971), in F.T.E.A., Leguminosae, Pap.: 757 (1971), Drummond in Kirkia 8: 226 (1972); Gonçalves in Garcia de Orta, Sér. Bot. 5: 107 (1982). *Dolicholus minimus* (L.) Hiern, Cat. Afr. Pl. Welw. 1: 267 (1896).

=*Rhynchosia minima* (L.). DC. var. *falcata* (E.Mey.) Verdc. in Kew Bull. 25:103 (1971) ***syn. nov***. *Copisma falcatum* E. Mey., Comm. Pl. Afr. Austr.: 136 (1836). Type: South Africa, Eastern Cape, *Inter Omtata et Omsamwubo in graminosis* [between Mthatha and Umzimvubu], alt. 1000-2000 ped., *Drége s.n.* (K!, lectotype, designated here). [Note: The specimen in K is chosen because it is the only one available. Since Meyer was based in P, one would have expected *Drége*'s specimens to be housed there, however, the specimen could not be located]

[Rhynchosia minima sensu Harv., Harv., Fl. Cap. 2: 254 (1862), *pro parte*; sensu Burtt Davy, Flow. Pl. Ferns Transvaal: 410 (1932), *pro parte*.]

Perennial climber (prostrate or erect) herb with long twinning or creeping stems. *Leaves* light green to bluish-grey; terminal leaflets 10–67×10–55 mm, obtuse, sharply acute to acuminate at the apex, glabrescent or sparsely puberulous; venation mostly paler and raised on the upper surface; with yellow to reddish glands on the lower surface, petiole length,10–60 mm long; stipules 1–5×0.5–1 mm, linear to narrowly ovate. *Inflorescences* 20–180 mm long. *Flowers* small, 3–8 mm long. *Calyx* tube 3–4 mm long, upper lobes 2.0–3.5 mm long, lower lobes 3–4 mm long, lateral lobes 1–3 mm long (Figure 2.8A). *Standard* 4–6×3 (4) mm (Figure 2.8B), wings 3–4×2 (Figure 2.8C), keel 4×2 mm (Figure 2.8D); ovary 2–3.5 mm long, covered with short whitish hairs and yellow glands (Figure 2.8F); style 2–5 mm long, sharp-curved and thick towards the tip (Figure 2.8F); stamen tube 5-6 mm long (Figure 2.8E). *Fruits* 10–20×3–5 mm, semi-falcate or falcate, narrowed at the base, very finely puberulous and glandular or almost glabrous, brown with some long bristly hairs. Flowering time: from spring through to late autumn (October–April).

Diagnostic characters: Leaves bluish-green, acuminate, with glands on the lower surface, glabrescent. Fruits are brown, covered with short and long bristly hairs, semi falcate or falcate (variable). *Rhynchosia minima* var. *minima* is morphologically similar to *R. minima* var. *glandularis*. *var.* nov., but can be distinguished from it by the colour (yellowish-green vs bluish-green) of the leaves and distribution of the glands on the leaf surface (glands on the lower surface only vs. glands on both sides).

In studying *Rhynchosia minima* var. *minima* and *R. minima* var. *falcata*, this study found that the two varieties are morphologically similar. The only difference between these two is the shape of the fruit. In *R. minima* var. *falcata*, the fruit is somewhat falcate whereas in *R. minima* var. *minima* it is mostly semi-falcate. The fruit is always linear-oblong (in

both varieties) when young and then curves as it matures. Therefore, this character cannot be used as a reliable taxonomic feature for diagnosis. Therefore, the two species are lumped together into *R. minima* var. *minima*.

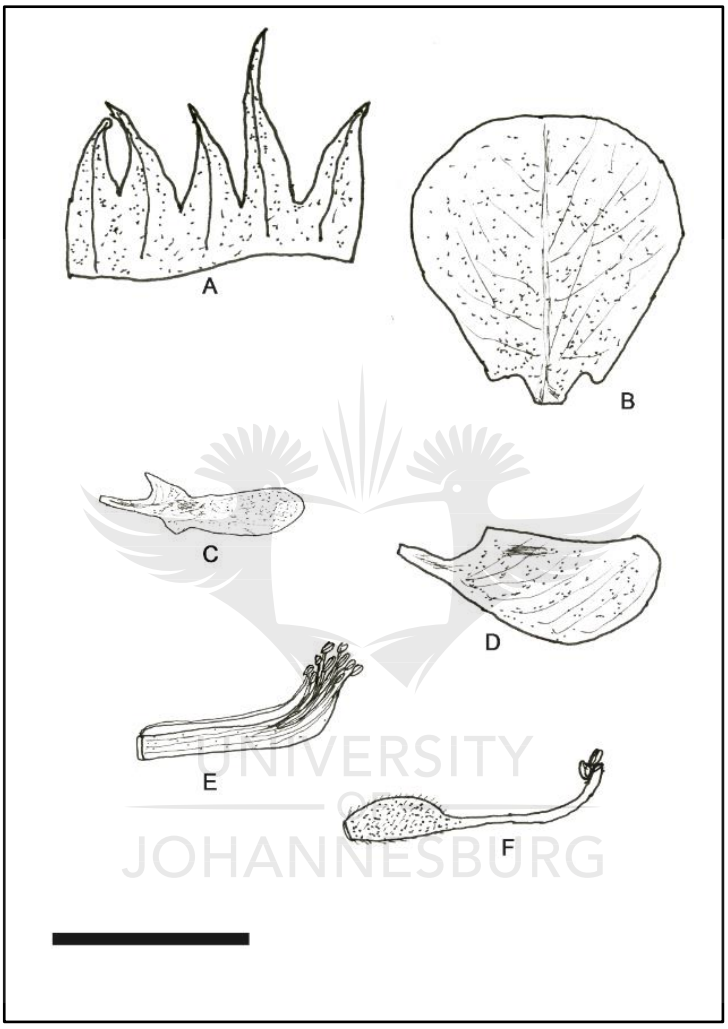

Figure 2.7: Floral morphology of *Rhynchosia minima* var. *minima*: A, calyx opened out with upper lobes to the left; B, standard petal; C, wing petal; D, keel petal; E, stamen sheath, showing nine stamens fused to stamen sheath and the vexillary stamen free; F, Gynoecium (ovary and style, with pollen on the stigma). Voucher specimen: *Le Roux 496* (PRE). Scale bar: A–F = 5 mm

Distribution and habitat: Within southern Africa, it occurs in Namibia, Botswana, South Africa (Limpopo, North West, Mpumalanga, Free State, and KwaZulu-Natal Provinces),

and eSwatini (Figure 2.8). In the rest of Africa, it occurs in Malawi, Mozambique, Zambia and Zimbabwe where it occurs in grassland, open mopane, woodland, grassy valleys (Verdcourt, 2001).

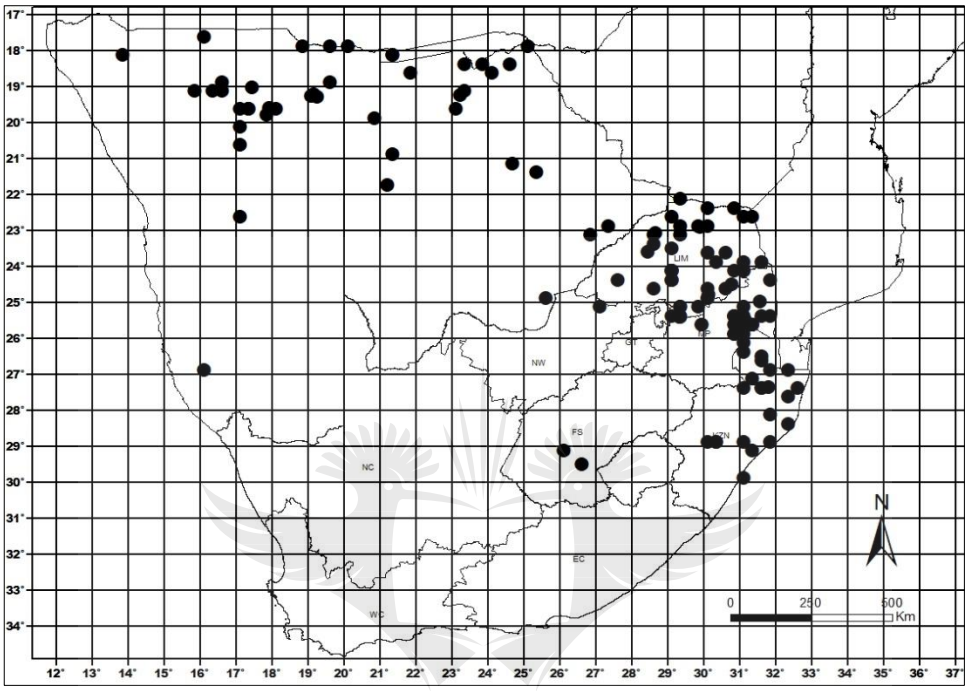

Figure 2.8: Known geographic distribution of *Rhynchosia minima var*. *minima* in southern Africa

Additional specimens examined

NAMIBIA: **1716** (Okavango): NW of Okavango, Uusivi (-CA), 21 December 1967, *Soini PRE56149* (PRE). **1718** (Kuring-kuru): 6 miles [9.65 km] E. of Tondoro Mission on road to Lupala (-DD), 16 December 1955, *De Winter 3969* (PRE). **1719** (Runtu): Kapako Camp, about 4 miles [6.4 km] W. of Mupini Mission (-DC), 07 February 1956, *De Winter and Marais 4505* (PRE); Okavango River banks, Runtu (-DD), 30 September *1949, Van Dam s.n.* (NBG). **1720** (Sambio): 3 miles [4.8 km] E. of Masari camp (-CC), 05 January

1956, *De Winter 4095* (PRE). **1813** (Ohopoho): Ohopoho, 3.5 miles [5.6 km] N of Ohopoho (-BB), 29 March 1957, *De Winter and Leistner 5257* (PRE). **1816** (Namutoni): 11 km *vanaf* [from] Otavi *op pad na* [on road to] Windhoek (-DC), 08 February 1974, *Grobbelaar 1885* (PRE). **1819** (Karakuwisa): Grootfontein North, Karakuwisa (-DC), 04 March 1958, *Merxmüller and Giess 1808* (PRE). **1821** (Andara): Dikundu, 19.2 km S. of Andara (-AB), 16 June 1971, *Giess 11428* (PRE); Roman Catholic Mission station camp (-AB), 24 April 1977, *Müller and Giess 516* (PRE). **1915** (Okaukuejo): On road to Ombica (-BB), 15 April 1973, *Le Roux 496* (PRE).**1916** (Gobaub): Etosha National Park, Charitsaub Flats, 5. of main road (-AB), 29 February 1976, *Giess and Loutit 14084* (PRE); Etosha National Park, Halali rest camp (-BA), 19 March 1984, *Retief 1430* (PRE). **1917** (Tsumeb): Farm Danevis-Süd GR/Ts 49 (-BC), 22 April 1968, *Giess 10376* (PRE); Oshikoto, Tsumeb, 39 km north of Tsumeb on B1 to Oshivelo (-AB), 12 April 1995, *Kubirske 203* (PRE); Grootfontein, Kumkauas Farm (-CA), 07 March 1974, *Merxmüller and Giess 30115* (PRE); Elefantenberg GR 584, between Elegantenberg and Vorberg (-CB); 04 February 1969, *Giess and Smook 10649* (PRE); Elefantenberg West GR 792 Farm (-CB), 30 January 1978, *Giess 14961* (PRE); Hoba Meteorite (-DB), 04 March 1995, *Germishuizen 7450* (PRE); on Rietfontein Farm on road to Otjituuo (-DD), 04 March 1995, *Germihuizen 7471* (PRE). **1918** (Grootfontein): near Grootfontein (-CA), 06 February 1934, *Schoenfelder 503* (PRE). **1919** (Kanovlei): Farm Talitha, on road to Verskyn (-AA), 08 March 1995, *Germihuizen 7745* (PRE); on road to Tsumkwe, 50 km NE of Grootfontein (-AC), 07 March 1995, *Germishuizen 7659* (PRE); on road to Tsumkwe, just after Vet Fence (-AD), 07 March 1995, *Germishuizen 7665* (-AD). **1920** (Tsumkwe): Betweeen Gautscha Pan and Nama Pan, 160 miles [257.5 km] E. of Grootfontein (-DD), 22 January

1958, *Story 6242* (PRE). **2017** (Waterberg): Otjinga (-AA), December 1935, *Boss TRV 35716* (PRE). Waterberg (-CA), May 1949, *Liebenberg 4756* (PRE). **2217** (Windhoek): Windhoek-Gobabis road, Avis Dam, 5 km E. of Windhoek (-CA), 06 March 1968, *Wanntorp and Wanntorp 56* (PRE). **2616** (Aus): Tsirub (-CC), 02 April 1934, *Dinter 7571* (PRE).

BOTSWANA: **1725** (Livingstone): Serondela, banks of Chobe River (-CC), 31 July 1950, *Robertson and Elffers 83* (PRE). **1821** (Andara): Tsodilo, Female Hill (-DB), 02 May 1975, *Biegel, Müller and Gibbs Russell 5067* (PRE). **1823** (Siambisso): 10 km N. of the Selinda Bridge on the road to the Kwando (-AD), 22 January 1978, *Smith 2207* (PRE); Northern district (-BD), 08 November 1974, *Smith 1170* (PRE). **1824** (Kachikau): Chobe, Serondela (-BC), June 1951, *Miller B/1162* (PRE); Zibadianja Lagoon, Savuti River near source (-CA), 19 October 1972, *Pope, Biegel, and Gibbs Russell 820* (PRE). **1923** (Maun): Okavango Swamp, Xhamoga Lediba Western most Island (-AB), 26 January 1974, *Smith 796* (PRE); Island in Boro Floodplain (-CA), 08 March 1973, *Biggs M55* (PRE); Mborogha floodplain, Island (-CA), 05 March 1975, *Biggs M686* (PRE). **2121** (Ghanzi): Groot Laagte Fossil river valley (-AD), 17 March 1980, *Smith 3214* (PRE); ± 25 km East of Ghanzi on road to Maun (-CC), 05 March 1996, *Burgoyne and Snow 5271* (PRE). **2124** (Rakops): Eastern Botswana along road from Francistown to Gaborone, 234. 4 km south of Francistown near Mopipi (-BA), 07 March 1996, *Burgoyne and Snow 5380* (PRE). **2125** (Tlada Mabeli): Lothlekane, Orapa Golf Course (-AD), 26 October 1974, *Allen 201* (PRE). **2227** (Palapye): Mabele a Pudi Hills (-CD), 12 March 1969, *Buerger 1181* (PRE). **2326** (Central District): Morale Pasture Research Station (-BB), 01

March 1977, *Hansen 3057* (PRE). **2425** (Gaborone): Between Manyana and Ramotswa along road (-DC), 31 March 1977, *Hansen 3108* (PRE).

SOUTH AFRICA, LIMPOPO PROVINCE: **2229** (Waterpoort): Musina (Messina) c.56 miles [90 km] N.W. *op plaas* [of the farm] Greefswald (-AB), 08 January 1974, *Pienaar 425* (PRE); ongeveer 20 km noordoos van Alldays op plaas Ceon (-CA), 25 March 1978, *Grobbelaar 2429* (PRE); Soutpansberg, Soutpan (-CD), 12 April 1934, *Schweickerdt and Verdoorn 473* (PRE); Northern side of Soutpansberg, farm Little Leigh (-DD), 03 March 1997, *Jordaan 3191* (PRE); Louis Trichardt ± 23 km N of Louis Trichardt in Wylies Poort (-DD), 05 April 1997, *Burgoyne 6230* (PRE). **2230** (Musina): Venda, Kupwe, tussen Madimbo en Segande (-BD), 04 February 1981, *Van Rooyen 3183* (PRE). **2231** (Phalaborwa): Kruger National Park, Klapperfontein (-CA), 19 February 1954, *van der Schijff 1724* (PRE); Wambia, Shilahlandonga *teenaan* [next] Mosambiek-Grens (-CB), 22 March 1979, *Grobbelaar 2379* (PRE). **2328** (Baltimore): Farm Reserve, ± 23 km N of Baltimore, at Steilloop turn-off ± 3km SE of Tolwe (-BA), 04 March 2004, *Jordaan 4218* (PRE); Calmar Ranch, Farm: Reserve, ca. 3 km directly SE from Tolwe (-BA), 04 March 2004, *Bester 4855* (PRE); R518 to Bakenberg (Road D192) about 5.7 km from Marken (-CB), 27 April 2016, *Jaca and Mkhize 849* (PRE). **2329** (Polokwane): Vivo (-AB), 20 January 1931, *Bremerkamp and Schweickerdt 217* (PRE); Manaka (-AB), 09 March 1894, *Schlechter 4636* (PRE). **2330** (Tzaneen): Moeketsi (-CA), April 1924, *Van Dam TRV 24998* (PRE); Letsitele, Janetsi Farm 463 LT on road Letsitele-Giyani (-CD), 21 May 1996, *Jacobsen 5289* (PRE). **2330** (Phalaborwa): 77 km from Tzaneen to Phalaborwa (-DA), 02 December 1975, *Stirton 5765* (PRE). **2331** (Phalaborwa): Skiettocht Military Base (-CC), 11 March 1991, *Manning 711* (PRE); 16 miles [25.74 km] S. of Letaba Camp (-

DC), 4 November 1948, *Codd & Dyer 4688* (PRE). **2427** (Thabazimbi): Geelhoutbosch Farm 269 KQ Part C. Drift near Rondawel Camp (-BC), 10 February 1996, *Retief and Strauss 2175* (PRE). **2428** (Modimolle): 15 miles [24 km] from Mokopane on road from Sterk river settlement, at bridge over the Mogalakwena River (-BB), 15 March 1971, *Coetzer 46* (PRE); Mookgophong, Mosdene (-DA), 20 February 1919, *Galpin M97* (PRE). **2429** (Zebediela): Mokopane (Potgietersrus), No specific locality (-AA), February 1904, *Bolus 11020* (BOL); Pyramid Estate, near Mokopane (-AA), 31 March 1921, *Galpin 8967* (PRE); Mokopane (-AA), 18 October 1909, *Leendertz 2328* (PRE); Mokopane (-AA), February 1904, *Bolus 11020* (PRE); Mokopane (-AA), 28 September 1908, *Leendertz 1293* (PRE); 9 km from Zebediela on road to Immer pan (-AC), 02 April 1972, *Clarke 386* (PRE); ± 9 km from Zedediela to Roedtan (-AC), 05 December 1975, *Stirton 5816* (PRE); West of Zebediela Estates, Groothoek 99 KS. (-AC), 26 March 1996, *Du Toit 5098* (PRE). **2430** (Pilgrim's Rest): Tzaneen-Ohrigstad Road 75 km S. of Tzaneen near Mica turn-off (-BA), 09 February 1982, *Brenan 14928* (NBG; PRE); Hoedspruit, Chester Farm (-BD), 02 February 1996, *Burgoyne 4111* (PRE); between Abel Erasmus Pass and Ohrigstad, about 15 km North of Ohrigstad in hills (-DA), 06 April 1994, *Burgoyne 2394* (PRE). **2431** (Phalaborwa): Phalaborwa Water board, 16 km south of Phalaborwa, near security kennels, farm Sheila 10-Ku (-AA), 05 September 1986, *Retief 458* (PRE); Phalaborwa Water Board, 14 km south of Phalaborwa, debushed area near evaporation banks (-AA), 22 July 1987, *Retief 607* (PRE); Kruger National Park, Olifants River, west of border along river (-AA), 05 April 1993, *van Rooyen and Bredenkamp 660* (PRE). **NORTH WEST PROVINCE**: **2527** (Rustenburg): Saulspoort on the mountain side (-AA), 29 November 1977, *Germishuizen 00516* (PRE). **MPUMALANGA PROVINCE**: **2429** (eMalahleni):

Hoewe H-71, *tussen* [between] Groblersdal and Marble Hall (-AB), 26 October 1963, Grobbelaar 24 (PRE). **2430** (Pilgrim's Rest): ±10 km from turn-off Steelpoort to Lydenburg (-CA), 11 December 2000, *Jordaan 3778* (PRE); Dwarsrivier valley, Steelpoort area (-CC), 18 May 1997, *PSG 36* (PRE); Spitskop area, about 2 km from Spitskop turn off riding south on Steelpoort/Lydenburg road, where Eskom powerlines cross the road obliquely (-CC), 02 April 1997, *Burgoyne 6014* (PRE); Sekhukhuneland, 5 km South West of Steelpoort (-CC), 12 October 2000, *Meyer 3049* (PRE). **2431** (Acornhoek): Hoedspruit-Phalaborwa road (-BD), 31 October 1981, *Zambatis 1240* (PRE); Skukuza Staff Village, garden of house no 135 (-DC), 12 March 1996, *Bengis BB1* (PRE). **2529** (eMalahleni): Hoew H-71, tussen Groblersdal and Marble Hall (-AB), 26 October 1963, *Grobbelaar 24* (PRE); just outside Groblersdal on road to Maleoskop, at show grounds (-AB), 16 March 1989, *Germishuizen 5230* (PRE); Kwa-Ndebele, farm Goederede (-AC), 16 March 1981, *Du Toit 208* (PRE); Loskop Dam Nature Reserve, near administration buildings (-AD), 16 February 1996, *Burgoyne and Snow 4590* (PRE); Loskop Dam (-AD), 14 October 1968, *Theron 1858* (PRE); 29 km from Stofberg on road to Roossenekal (-BB), 01 November 1977, *Gernishuizen 416* (PRE); ± 10 km WSW from Belfast, Langkloof Farm (-DB), 30 January 1996, *Burgoyne 3975B* and *3984* (PRE). **2530** (Mashishing): Lowveld Botanic Garden, garden side, central area (-BD), 06 November 1970, *Buitendag 752* (PRE); Lowveld Botanic Garden (-BD), 6 November 1970, *Buitendag 752* (NBG); Barberton (-BD), March 1931, *Liebenberg 2330* (PRE); Kaapsche Hoop (-DB), March 1918, *Rogers 20779* (PRE). 5 km from Barberton on road to Mbombela (Nelspruit) (-DD), 09 March 1989, *Germishuizen 5091* (PRE); 60 km from Badplaas on road to Barberton (-DD), 09 March 1989, *Germishuizen 5088* (PRE). **2531** (Komatipoort): 2 km W. of Sabie River

bungalows on Sabie Road (-AA), 07 April 1971, *Stephen 330* (PRE); 14 km from Mbombela on road to Komatipoort (-AC), 19 February 1977, *Stirton 6772* (PRE); Thanda-Nani Game Reserve, about 10 km from Hectorspruit on road to Malelane (-BC), 04 December 1999, *Germishuizen 9991* (PRE); At Lebombo, Komati River (-BD), 11 March 1989, *Germishuizen 5151* (PRE); 10 km from Barberton on road to Kaapmuiden (-CA), 10 March 1989, *Germishuizen 5121* (PRE); ± 3 km from Mbombela-Kaapmuiden T-Junction on road to Mara (-CA), 04 March 1999, *Meyer 1910* (PRE); 3 km from Barberton on road to Kaapmuiden (-CA), 10 March 1989, *Germishuizen 5113* (PRE); 4 km from Barberton on road to Kaapmuiden (-CA), 10 March 1989, *Germishuizen 5117* (PRE); Barberton phase 2 Mundt's Concession (-CA), 29 November 1998, *Williamson 849* (PRE); Louws Creek (-CA), October 1922, *Wager TRV 22456* (PRE); Kaapmuiden, 2 km from Kudu Lodge on road to Komatipoort (-CB), 11 March 1989, *Germishuizen 5125* (PRE); Kaapmuiden (-CB), (Precise date not provided) December 1921, *Rogers 25074* (PRE); 10 km from Kaapmuiden on road to Mbombela, Crocodile River Gorge, 27 December 1996, *Germishuizen 8713* (PRE); 27 km on Kaapmuiden road from Barberton turn-off (-CB), 15 August 1985, *Germishuizen 3295* (PRE); 30 km from Kaapmuiden on road to Komatipoort (-CB), 19 February 1977, *Stirton 6798* (PRE); Barberton (-CC), December 1916, *Pott 5316* (PRE); valley near Edwin Bray Battery, 26 November 1890, *Galpin 1196* (PRE). **FREE STATE PROVINCE**: **2926** (Bloemfontein): *Onder brug langs* [under the bridge along] N1 *Nasionale pad* [National road] (-AA), 26 March 1993, *Malan 449* (PRE); Lambons Plantation (-No specific grid), March 1917, *Potts 2916* (PRE). **KWAZULU-NATAL PROVINCE**: **2632** (Bela Vista): Mavilo Hill, Pongola flood-plain

(-CD), 04 November 1969, *Moll 4277* (PRE). **2731** (Louwsburg): Itala Nature Reserve, ca. 2 miles [3.2 km] from Bivane-Pongola junction on track to Warden's House (-AC), 08 January 1976, *Brown and Shapiro 366* (PRE); Pongola town, at canal (-BC), 21 October 2001, *Meyer BP00963* (PRE); Pongola area on the road from Golela to Pongola near junction to Sodwana/Mkunzi/Pongola, ± 300 m from junction (-BD), 18 February 1996, *Burgoyne and Snow 4601* (PRE). **2732** (Ubombo): 3 miles [4.8 km] east of Pongola River on road to Maputa (-AB), 21 November 1969, *Moll 4506* (PRE); Maputaland, Lake Sibaya, next to road along eastern edge of Sibaya (-BC), 02 September 1996, *Felton and Thornhill 139* (PRE); Manzengwenya Forestry Station (-BC), 19 December 1974, *Vorster 2604* (PRE); Mkuzi Game Reserve (-CB), 10 March 1991, *Steiner 2277* (PRE). **2830** (Dundee): 12 miles [19.3 km] WNW of Muden (-CC), 15 March 1947, *Acocks 13467* (PRE); 11 km from Keate's drift on road to Muden (-CD), 22 January 1980, *Arnold 1384* (PRE); Muden, to W. of Muden near town (-CD), 15 March 1947, *Acocks 13474* (PRE). **2831** (Nkandla): Hlabisa, Hluhluwe Nature Reserve (-BB), 19 April 1955, *Ward 2543* (NU; PRE); Umhlatuzi Valley, Ngoye (-DD), 31 May 1967, *Venter 3725* (PRE). **2832** (Mtubatuba): Maphelane Nature Reserve, railway camp (-AD), 15 October 985, *Fokkens 15* (NH; PRE); Umhlatuzi Lake Bluff (-CC), 15 February 1968, *Venter 4582* (PRE). **2930** (Pietermaritzburg): Tongaland, Umhbanga Nek (-CB), 10 December 1964, *Bourquin 367* (NU). **2931** (Stanger): North bank of Tugela River mouth (-AB), 13 October 1975, *Stirton 5356* (PRE); Durban (-CC), 19 April 1947, *Medley-Wood 6383* (PRE).

ESWATINI: **2631** (Mbabane): Bulunga Point (-AA), 20 March 1964, *Compton 32007* (NBG; PRE); Hhohho, 20 km from Piggs Peak, at Old Bridge over Nkomati River (-AA), *Germishuizen 6036* (PRE); 24 miles [38.6 km] from Manzini on road to Siteki (-AC),

24 October 1971, *Clarke 274* (PRE); Hlatikulu, Big Bend (-DD), 30 October 1961, *Compton 30898* (NBG); No specific locality, July 1910, *Stewart TRV 8943* (PRE).

2.3.1.5. ***Rhynchosia minima*** (L.) DC. var. ***prostrata*** (Harv.) Meikle in Kew Bull. 9: 275 (1954); in F.W.T.A., ed. 2, 1: 555 (1958); Torre in Consp. Fl. Angol. 3: 311 (1966); Verdcourt in Kew Bull. 25: 104 (1971); in F.T.E.A., Leguminosae, Pap. : 758, fig. 109/42 (1971); Drummond in Kirkia 8: 226 (1972). *Rhynchosia memnonia* var. *prostrata* Harv. in Fl. Cap. 2: 253 (1862); Eyles in Trans. Roy. Soc. South Africa 5: 382 (1916); Baker f., Legum. Trop. Africa: 473 (1929). In Fl. Cap. 2: 253 (1862). Type: South Africa, Gauteng Province, Magaliesberg, *Zeyher 504* (K! holotype).

=*Rhynchosia hockii* De Wild. in Repert. Spec. Nov. Regni Veg. 11: 541 (1913); Hauman in Fl. Congo. Belge 6: 164, t. 13 (1954). Type: Democratic Republic of Congo, Guba, Ober-Katanga, *Hock A. s.n.* (BR! holotype).

=*Rhynchosia hockii* var. *grandifolia* Hauman in F.C.B. 6: 165 (1954); in Bull. Jard. Bot. Brux. 25: 109 (1955). Type: Democratic Republic Congo, *District des Lacs Édouard et Kivu, Bukavu*, *Gilon 203* (BR!, holotype).

=*Rhynchosia ischnoclada* Harms in Notizbl. Bot. Gart. Berlin-Dahlem 12: 513 (1935). Type: Tanzania, *Bezirk Lindi: Etwa 40 km westlich von Lindi, 210 m ü. M., Lutamba-See, Ufer-Umgebung*, *Schlieben 5614* (B!, holotype).

Perennial prostrate herb with long trailing, pubescent silvery stems*. Leaflets* 10–34×10–35 mm, mostly round at the apex, densely pubescent to velvety; glands on the lower surface of the leaf; petiole length up to 60 mm long; stipules lanceolate, 2–5×0.5–1 mm, linear to narrowly ovate. *Inflorescences* axillary racemes, 20–130 mm long. *Flowers* large, 4–10 mm long. *Calyx* tube 4 mm long, upper lobes 3–5 mm long, lower lobes 4–5 mm

long, lateral lobes 2–3 mm long (Figure 2.10A). *Standard* 5–7×±5 mm (Figure 2.10B), wings ±4×1–2 (Figure 2.10 C), keel 4–6×±2 (3) mm (Figure 2.10D); ovary 2–3 mm long, covered with short dense white hairs and very few yellow glands (Figure 2.10F); style 4–7 mm long, sharply curved and thick at the end (Figure 2.10F); stamen tube 6–9 mm long (Figure 2.10E). *Fruits* 10–22×3–6 mm, brown, covered with short and long hairs and glands. Flowering time: from early spring through to late autumn (August–April).

Diagnostic characters: *Rhynchosia minima* var. *prostrata* resembles *R. minima* var. *memnonia* in habit, pubescence, and flower size. It differs from *R. minima var. memnonia* in that the leaflets of *R. minima* var. *prostrata* are smaller (10–30×10–35 mm) than those of *R. minima* var*. memnonia* (14–36×17–35). Furthermore, it has a longer petiole (10–60 mm in *R. minima* var. *prostrata* versus 20–35 mm in *R. minima* var. *memnonia*).

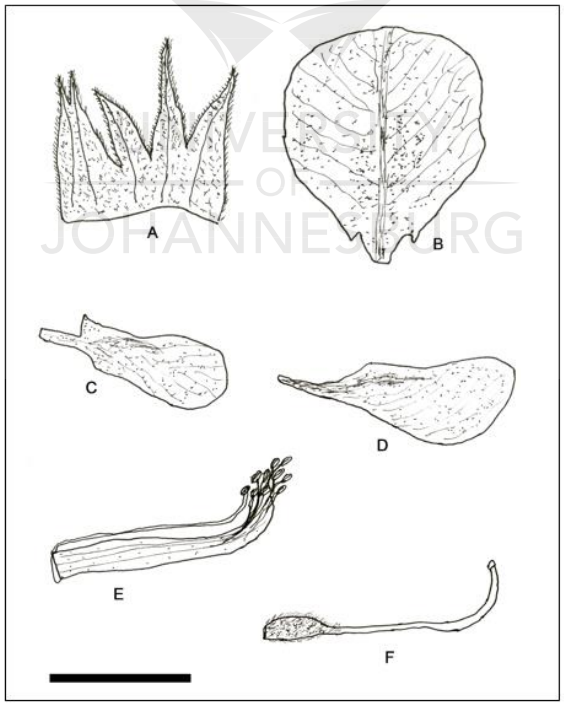

Figure 2.9: Floral morphology of *Rhynchosia minima* var. *prostrata*: A, calyx opened out with upper lobes to the left; B, standard petal; C, wing petal; D, keel petal; E, stamen sheath, presenting nine stamens fused to stamen sheath and the vexillary free. Voucher specimen: *Green 501* (NH). Scale bar: A–F = 5 mm

Distribution and habitat: Within southern Africa, this variety occurs in Namibia, Botswana, and South Africa (Limpopo, North West, Gauteng, Mpumalanga, Free State, KwaZulu-Natal and Eastern Cape Provinces) (Figure 2.11). It also occurs in Malawi, Mozambique, Zambia and Zimbabwe in grassland, open mopane woodland, and roadsides; with altitude range: 450–1500 m. It is also found in drier parts of tropical Africa (Verdcourt, 2001).

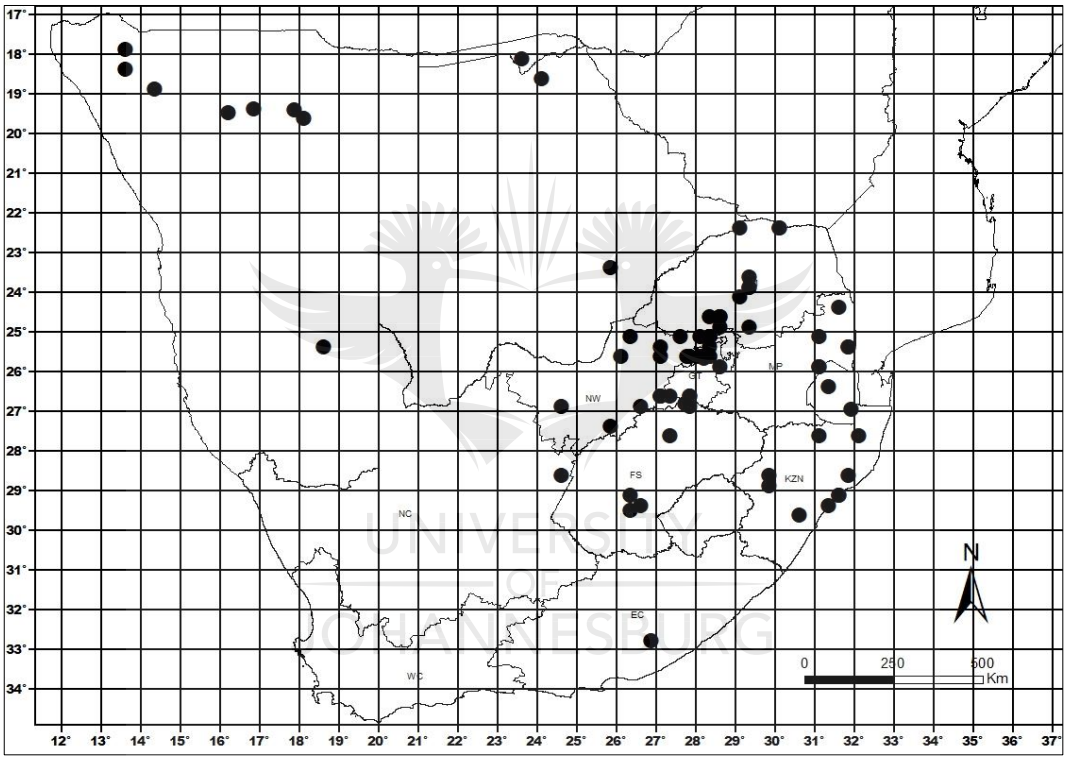

Figure 2.10: Known geographic distribution of *Rhynchosia minima var prostrata* in southern Africa

Additional specimens examined:

NAMIBIA: **1713** (Swartbooisdrif): Ombazu, 11 km from town (-DC), 10 April 1973, *Giess and Van der Walt 12681* (PRE).**1813** (Ohopoho): Kaokoveld Reserve, Kaoko Otavi (-BC),

3 miles [4.82] NE of town, 17 April 1957, *De Winter and Leistner 5543* (PRE). **1814** (Otjitundua): Kaokoveld, Otjitjekwa, 9 km S. of town (-CD), 28 April 1966, *Giess 9421* (PRE).**1823** (Singalamwe): Caprivi Strip, E. of the Cuando River (-BA), October 1945, *Curson 1220* (PRE). **1916** (Gobaub): Tsumeb, Nature Conservation & Tourism, Etosha Game Park, 5 miles [8 km] N. of South boundary opposite Vrede Farm (-BD), 19 February 1966, *Tinley 1320* (PRE); North of Outjo, Ryno Farm (-CA), *Germishuizen 9720* (PRE); Oliewenhof Farm (-CA), March 1974, *Merxmüller and Giess 30130* (PRE). **1917** (Tsumeb): At junction of tar road between Otavi and Grootfontein (-BD), 3 March 1995, *Germishuizen 7441* (PRE).**1918** (Grootfontein): Grootforntein (-CA), 23 January 1934, *Schoenfelder S491* (PRE). **2518** (Tses): Asis (-BC), 08 January 1939, *Screiber 698* (PRE).

BOTSWANA: **1824** (Kachikau): Chobe National Park, Savuti, along and at foot of rocky hill, at fringe of woodland (-CA), 25 March 1984, *Jacobsen 3038* (PRE). **2325** (Lephephe): Lephephe village, 16 km NW of village on Kalahari sandveld past reserve station (-BD), April 1969, *Kelaole 584* (PRE).

SOUTH AFRICA, LIMPOPO PROVINCE: **2229** (Waterpoort): Breslau (-AC), 10 September 1986, *Straub 371* (PRE). **2230** (Musina): Musina (-AC), September 1918, *Rogers 21839* (PRE). **2329** (Polokwane): Moletsi, at a small stream near the windmill, 1 km from Moletsi Nature Reserve (-CB), April 1995, *Makgakga 118* (PRE); Polokwane Nature Reserve, *rooi grasveld naby hek* [red grassland near the gate] (-CD), 07 February 1980, *Bredenkamp 375* (PRE); Polokwane Nature Reserve (-CD), 12 January 1979, *Bredenkamp* and *Van Vuuren 300* (PRE). **2428** (Modimolle): Mokopane, Nyl River, near great road to Mokopane (-CB), 17 February 1964, *Oakes and Scheepers 260* (PRE);

Mookgophong (Naboomspruit), 4 miles [6.43 km] from town on road to Crecy (-DA), 17 March 1972, *Clarke 363* (PRE); Mookgophong, Mosdene (-DA), 22 January 1919, *Galpin M93* (PRE); Mookgophong, Mosdene, Vogelstruispan and Geluk (-DA), 19 March 1923, *Galpin M584* (PRE); Settlers, outskirts of village (-DC), 05 November 1972, *Clarke 409* (PRE). **2429** (Zebediela): Mokopane (-AA), 28 September 1908, *Leendertz 1293* (PRE); Marble Hall, Hoewe H-71 (-CD), 30 December 1965, Grobbelaar 468 (PRE). **NORTH WEST PROVINCE**: **2526** (Zeerust): Marico, Rooikoppiesfontein Farm (-AB), 11 March 1970, *Carter 949* (PRE); Zeerust (-CA), January 1912, *Leendertz 4363* (PRE). **2527** (Rustenburg): Pilanesberg, Houwater, Houwater 54 JQ Farm near dam (-AC), *Germishuizen and Retief 663* (PRE); Rustenburg, 3 miles [4.8 km] from town on road to Thabazimbi (-BA), 27 March 1967, *Grobbelaar 449* (PRE); Rustenburg Townlands Commonland, North Eastern Asiatic Residential area (near boundary fence) (-CA), 19 February 1970, *Scheepers 9* (PRE); Bokfontein (-DB), December 1908, Jenkins *TRV6916* (PRE); Brits, ca 2 km E. along road to Ga-Rankuwa (-DB), 16 February 1976, *Van der Meulen 724* (PRE); Britz (-DB), 20 November 1995, *Rheinheimer RH7* (PRE). **2624** (Vryburg): Vryburg (-DC), 11 April 1921, *Mogg 8925* (PRE). **2626** (Klersksdorp): Convent of the Sacre (-DC), 15 November 1927, *De Victoria 25* (PRE). **2627** (Potchefstroom): Frederickstad (-CA), 18 December 1944, *Louw 1303* (PRE); Boskop (-CA), 18 December 1939, *Louw 589* (PRE); 1 km out of Potchefstroom on road to Parys just before Loopspruit (-CA), 09 February 1981, *Ubbink 1056* (PRE); Klipdrift (-CB), 09 January 1935, *Theron 1231* (PRE); 39 miles [62.7 km] S. of Johannesburg on road to Sasolburg (-DB), 04 February 1971*, Grobbelaar 1413* (PRE); Eligwa Boat Club, about 2 km E. of Baddrif Bridge on Vaal River, alongside foot path (-DB), 06 April 1994, *Kroon*

11361 (PRE). **2725** (Bloemhof): Wolmaransstad, Oersonskraal Farm (-BD), 03 December 1928, *Sutton 82* (PRE). **GAUTENG PROVINCE**: **2528** (Pretoria): Bela-Bela (Warmbaths), just outside Bela-Bela on road to Pretoria (-AA), 11 March 1978, *Germishuizen 800* (PRE); Codrington Station, 2 miles [3.21 km] of station (-AB), (precise date not provided), *Codd 1032* (PRE); Codrington station, near station on Pretoria-Bela-Bela road (-AB), 11 March 1946, *Codd 901* (PRE); Pienaars River, Middelkop Farm near Pienaars River (-AB), 20 January 1926, *Smith 2137* (PRE); Bela-Bela, 18 km from Bela-Bela to Pretoria (-AB), 08 February 1982, *Stirton 10619* (PRE); 3 km from Radium to Bela-Bela (-AB), 06 February 1981, *Stirton 10545* (PRE); Bela-Bela, Bothasvlei Nature Reserve (-AB), 11 April 1994, *Van Wyk 12336* (PRE); North of Hammanskraal on road to Bela-Bela (-AD), 14 October 1996, *Germishuizen 8683* (PRE); Doornpoort, Second extension, drainage line crossing Amandelboom Road (-CA), 01 November 2003, *Bester 4291* (PRE); Sinoville, field bordering the southern border of the Wonderboom Airport (-CA), 17 January 2004, *Bester 4642* (PRE); Swartspruit (-CA), 27 October 1919, *Pole-Evans 404* (PRE); Villeria (-CA), 19 December 1919, *Mogg 7323* (PRE); Just outside Pretoria on road to Bela-Bela (-CA), 25 February 1979, *Germishuizen 1052* (PRE); Pyramid (-CA), 01 November 1915, *Mogg PRE11627* (PRE); Onderstepoort (-CA), 07 November 1912, *Theiler PRE56157* (PRE); 12 October 1912, *Theiler 9305* (PRE); Onderstepoort, side of target pit of shooting butts (-CA), 25 February 1932, *Smith 6068* (PRE); October 1917, *Mogg 462* (PRE); North of Wonderboom Airport, Cynthiavale Agricultural Small Holdings (-CA), 20 October 2005, *Bester 6040* (PRE); Wonderboom Poort (-CA), 02 November 1905, *Leendertz 655* (PRE; BOL); Baviaanspoort, bank of Pienaars River near Outspan (-CB), 28 October 1925, *Smith 1072* (PRE); Koedoespoort,

N side of hill (-CB), 06 December 1925, *Smith 1613* (PRE); Willow Glen (-CB), 02 December 1959, *Strey SKF883* (PRE); Doornpoort, drainage line crossing Amandelboom Road (-DA), 05 December 2003, *Bester 4450* (PRE); Doornpoort, Dr Swanepoel Road, northern side of N4 Highway, near Doornpoort Toll Plaza (-CA), 10 May 2018, *Mothogoane 850B* (PRE). **2528** (Johannesburg): Kaalfontein (-DC), 24 December 1918, *Pole-Evans PRE18913* (PRE). **MPUMALANGA PROVINCE**: **2431** (Acornhoek): Kruger National Park (-BC), March 1975, *Gertenbach 5408* (PRE). **2531** (Komatipoort): Kruger National Park, Pretorius Kop, My Acre of Africa (-AB), 21 January 2015, *Zambatis GZ1065* (PRE); Komatipoort, Kruger National Park, Pabid (-AA), 23 January 1954, *Van der Schijff 3515* (PRE); Komatipoort (-BD), 15 December 1897, *Schlechter 11750* (PRE); Komatipoort (-BD), November 1913, *Rogers 12944* (PRE); 1 km from Barberton on road to Havelock (-CC), 10 March 1989, *Germishuizen 5099* (PRE); 4 km from Barberton on road to Mbombela (-CC), 13 August 1985, *Germishuizen 3229* (PRE). **FREE STATE PROVINCE**: **2627** (Potchefstroom): Uitkmost Farm 413, as crow flies 5-6 km West, North West of Sasolburg, on summit of koppie between rocks (-DC), 18 December 1996, *Kroon 14057* (PRE); Sasol Game Park, Wilgefontein, boundary Parys/Sasolburg road, 50 km ex entrance to Sasolburg (-DD), 06 November 1995, *Kroon 11725* (PRE). **2727** (Kroonstad): Kroonstad (-CB), January 1929, *Pont 166* (PRE). **2926** (Bloemfontein): Van Tonder (-AB), 28 December 1911, *Burtt Davy 11749* (PRE); Wilgenhof, W. of Daniel *van* [from] Niekerk Street (-AB), 25 February 1967, *Hanekom 816* (PRE); Rustfontein Dam, reserve at dam (-BC), 28 October 1991, *Engelbrecht JE531* (PRE). **KWAZULU-NATAL PROVINCE**: **2732** (Ubombo): Ingwavuma, Ndunu Game Reserve (-AA), 22 December 1971, *Pooley 1615* (NU); Ngotshe, Jozini Dam, road between Mkuze and Candover, West

slope of Lebombo mountains (-CA), 27 April 1964, *Ross 1125* (NU; PRE); Jozini Dam, Mountain Pass near dam (-CA), 13 December 1973, *Stirton 489* (PRE); ± 3 miles [4.8 km] from Mkuze on road to Nongoma (-CB), 26 April 1964, *Gordon-Gray 4717* (NU); Mkuzi Game Reserve, Mbonyona, Stand 54 (-CB), 28 March 1977, *Goodman 962* (NU). **2829** (Harrismith): Colenso commonage, W. of the highway on Winterton Road (-DB), 14 February 1988, *Green 501* (NH); Frere, 11 miles [17.7 km] N. of Estcourt (-DD), 01 May 1964, *Ross 1179* (PRE). **2831** (Nkandla): Heatonville, Thorncreek (-AB), 02 September 1963, *Venter 1178* (PRE). **2832** (Mtubatuba): Hluhluwe Game Reserve (-AA), 05 November 1983, *Phelan 736* (NU). **2930** (Pietermaritzburg): Plateau, Ukulinga Research Farm (-CB), 06 November 2004, *Wragg 873* (NU); near Roy Hesketh Circuit, Cleland (-CB), October 1962, *Morris 18* (NU); Oribi Airfield-Railway line (-CB), 24 October 1957, *Brayshaw 85* (NU); Cato Ridge, inland of Durban (-DA), 19 September 2013, *Styles 4364* (NH); Ingamankulu Hill, Hope Farm (-DB), 09 September 2007, *Young 557* (NU). **2931** (Stanger): Mtunzini, Gingindlovu (-BA), 22 October 1950, *Lawn 1774* (NH); Lower Tugela (-BA), 27 March 1964, *Ross 861* (NU; PRE); Mtunzini, Gingindlovu (-DD), 24 September 1945, *Acocks 11815* (NH; PRE). **NORTHERN CAPE PROVINCE**: **2824** (Kimberley): veld by hills, Newlands (-AD), March 1934, *Wilman 3029* (BOL); Spitzkop (-DA), February 1937, *Acocks 1803* (PRE). **EASTERN CAPE PROVINCE**: **3226** (Fort Beaufort): Fort Hare, Honeydale Farm, WSM, Trollope's Burn-Browse plots, Goat Plot (-DD), 08 December 1976, *Gibbs Russell 3111* (PRE).

ESWATINI: **2631** (Mbabane): Umvoloosi River (-AD), January 1906, *Bolus 11846* (PRE); ± 10 km from Big Bend on the road to Lavumisa, West of Lebombo Mountains, between the tarred road and the river (-DD), 18 February 1996, *Burgoyne and Snow 4600* (PRE).

CHAPTER 3: SYNOPSIS OF SELECTED MEDICINAL RHYNCHOSIA SPECIES IN SOUTHERN AFRICA

3.1. Introduction

The taxonomy of several *Rhynchosia* species in the type section remain uncertain and require further study, however, a taxonomic revision of the type section is beyond the scope of the current study. The aim of this study is to provide a synopsis of only the ethnomedicinally important species of the genus, six of which belong to *Rhynchosia* sect. *Rhynchosia*. Seven species of *Rhynchosia* are used traditionally for medicinal purposes, these are: *R. adenodes* Eckl. & Zeyh., *R. albissima* Gand., *R. caribaea* (Jacq.) DC., *R. harveyi* Eckl. & Zeyh., *R. hirta* (Andrews) Meikle & Verdc., *R. resinosa* (A. Rich.) Baker and *R. sublobata* (Schumach.) Meikle (Kokwaro, 1976; Hedberg et al., 1983; Gelfand et al., 1985; Anokbonggo et al. 1990; Dold and Cocks, 1999; Shale et al., 1999; Neuwinger, 2000 ; Mageresi et al., 2007; Ssegawa and Kasenene, 2007; Jeruto et al., 2010; 2011; Moffett, 2010; Bruschi et al., 2011; Moteetee and Van Wyk, 2011; Stark et al., 2013; Barik et al., 2014; Lima et al., 2014; Amjad et al., 2015; Possa and Khotso, 2015). This synopsis provides correct typification and nomenclature of the species as well as brief descriptions and their distribution patterns.

3.2. Materials and methods

The same procedures were followed as in chapter 2.

3.3. The Synopsis

3.3.1. ***Rhynchosia adenodes*** Eckl. & Zeyh., in Enum. Pl. Afr. Austral: 254 (1836); Harv. in Harv. & Sond. Fl. Cap. 2: 254 (1862).

=*R. amatymbica* Eckl. & Zeyh, in Enum. Pl. Afr. Austral: 254 (1836).

Perennial, erect or spreading herbs with long trailing stems (0.1–0.6 m long), growing from a woody rootstock. *Stems* procumbent, glabrous or pubescent. Terminal leaflets 6–40 × 5–30 mm, ovate-orbicular or sub-rhomboid, acute or obtuse, resinous red gland dots beneath; petiole 5–35 mm long; stipules ovate-lanceolate, at right angles to the stem, glabrous, 2–7×0.5–2.5 mm. *Inflorescence* in axillary racemes or terminal clusters, 4–14 cm long, glabrous-pubescent, exceedingly longer than the leaves. *Flowers* 7–14 mm long, yellow with orange or maroon/purple venation on petals, glabrous or pubescent, glandular, clustered towards the tips of the inflorescences. *Fruits* 15–18×4–6 mm, glabrous or pubescent, glandular. Flowering time: from early spring through to mid-autumn (September—March).

Diagnostic characteristics: *Rhynchosia adenodes,* closely resembles *R. cooperi* (Harv.ex Baker) Burtt Davy in growth form and habitat, as well as leaf shape (ovate-orbicular) and flower colour (yellow), but it can be distinguished by the red tiny glands on the lower surface of the leaves, as opposed to *R*. *cooperi* in which the glands are lacking on the leaves. The petiole in *R. adenodes* is much longer (5–35 mm) than in R*. cooperi* (5–15 mm).

Distribution and habitat: *Rhynchosia adenodes* has a restricted distribution in southern Africa, from South Africa (Limpopo, North West, Gauteng, Mpumalanga, Free State, KwaZulu-Natal, Western Cape and Eastern Cape Provinces) and Lesotho (Figure 3.1). It is found in grassland, forest, among rocks in hillsides.

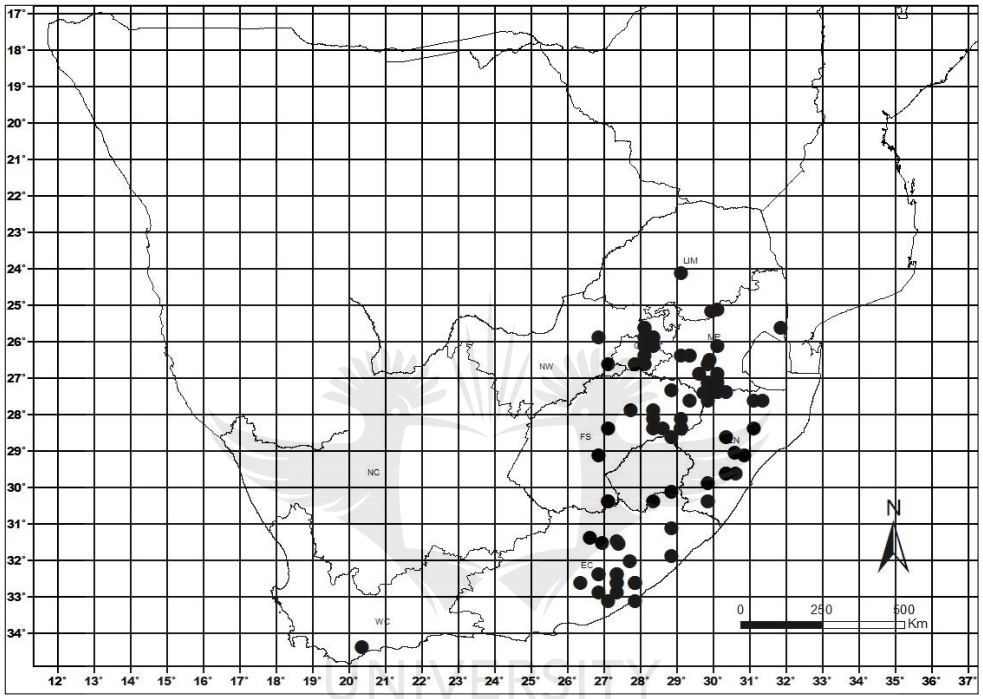

Figure 3.1: Known geographic distribution of *Rhynchosia adenodes* in southern Africa

Conservation status: LC (Raimondo et al., 2009)

Additional specimens examined

SOUTH AFRICA, LIMPOPO PROVINCE: 2429 (Zebediela): Waterberg District, Mokopane (-AA), (Precise date not provided), *Crawley PRE 55980* (PRE). **NORTH WEST PROVINCE**: **2526** (Zeerust): Marico District, Koster Station (-DD), 12 November 1907, *Burtt-Davy 7172* (PRE). **2627** (Potchefstroom): Pienaarskamp (-CA), 09 November 1982,

Ubbink 1122 (PRE). **GAUTENG PROVINCE**: **2528** (Pretoria): Fairy Glen (-CA), 06 October 1933, *Mogg 14139* (PRE); At Rosslyn extension on road to Brits (-CA); 16 October 1996, *Germishuizen 8705* (PRE); Pinedene, near Irene (-CC), 27 September 1904, *Burtt-Davy 2323* (PRE); Faerie Glen Nature Reserve (-CD), 01 March 2018, *Mothogoane & Masupa 06* (PRE) Van Riebeeck Natuur Reservaat (-CD), 17 October 1968, *Kok 275* (PRE); Rietvlei Dam (-CD), November 1935, *Obermeyer TRV 34848* (PRE). **2627** (Potchefstroom): Meyerton, Daleside, Walker's Fruit Farms Plots 21-23 (-DB), 13 October 1979, *Mitchley 46* (PRE); Vereeniging, Leeuwkuil Pasture Research Station (-DB), 05 November 1935, *Story L48* (PRE). **2628** (Johannesburg): Witwatersrand, Forest Hill (-AA), (Precise date not provided), 1921, *Rogers 24327* (PRE); Geduld (-AB), 22 October 1927, *Young & Young 2023* (PRE); Witwatersrand, Palmietfontein (-AC), 06 November 1952, *Gilliland 26809* (PRE); Heidelberg District, Kapperskraal (-AD), 12 November 1916, *Burtt Davy 17119* (BOL); Johannesburg, Suikerbosrand (-CA**)**, 16 October 1971, *Bredenkamp 135* (PRE). **MPUMALNGA PROVINCE**: **2529** (eMalahleni): Mapoch Cave ca.7 km NE of Roossenekal (-BB), 18 January 2005, *Maserumule 203* (PRE). **2530** (Lydenburg): N.E. of foothills of Steenkampsberg (-AA), 19 November 1933, *Young A469* (PRE); Lydenburg District, Sabie valley (-AB), 27 March 1933, *Galpin s.n.* (BOL). **2531** (Komatipoort): Komati River (-DB), November 1907, *Nicholson PRE 4598* (PRE). **2629** (Bethal): Bethal, 3.3 miles [5.31 km] from Kinross to Trichardt (-AC), 07 November 1966, *Marsh 108* (PRE); Bethal (-AD), December 1910, *Leendertz 3584* (PRE); Bethal, 2.5 miles [4.02 km] E. of Bethal (-AD), 20 November 1946, *Codd 2163* (PRE); Ermelo (-DB), October 1913, *Collins TRV 13232* (PRE); Ermelo District: Derdepoort farm (-DB), 16 November 2010, *Bester 10398*

(PRE); Bethal, 15 km from Morgenzon on the road to Paul Kruger Bridge (-DC), 27 September 1977, *Venter 2064* (PRE). **2630** (Carolina): Carolina, Bergendal farm (-AA), 20 October 1932, *Galpin 12500* (BOL; PRE); Kalkoenkranz, Elandsberg farm (-CC), 06 November 1984, *Turner 317* (PRE). **2729** (Volksrust): Ermelo District, Amersfoort, Roodekoppies farm (-BB), 28 October 1986, *Turner 1121* (PRE); Welgedacht farm (-BB), 01 November 1986, *Turner 1166* (PRE); Volkrust (-BD), 07 October 1906, *Kamering TRV 5707* (PRE); Amersfoot, on farm Maquiabe, in hills behind the main dam which lies in valley below the house (-BB); Volksrust District: Farm Waterval, Tierkloof 4 x 4 route (-BC), 02 December 2015*, Steyn 2171* (PRE). **2730** (Vryheid): Groot Vlei (-AA), November 1961, *Jacobsz 138* (PRE); Wakkerstroom, North Hill summit (-AC), 20 January 1930, *Galpin 10219* (PRE); Wakkerstroom District, Honeymoon Kloof (-AC), 20 November 1916, *Beeton 68* (PRE); Wakkerstroom District, Oshoek (-AD), 30 October 1963, *Devenish 1057* (PRE). **FREE STATE PROVINCE**: **2727** (Kroonstad): Lindley District, ca. 20 km ENE of Steynsrus (-DC), 15 November 2008, *Bester 8554* (PRE). **2728** (Frankfort): Between Frankfort and Vrede (-BD), 08 December 2000, *Meyer BP00388* (PRE); Sweet home-20 km west of Reitz on Bulthoek road (-CD), 26 January 1983, *Retief 965* (PRE). **2729** (Volksrust): 20 km from Memel on road to Vrede (-CB), 09 March 1987, *Germishuizen 4357* (PRE). **2827** (Senekal): Winburg, Willem Pretorius Wildtuin (-AC), 12 January 1977, *Muller 1982* (PRE). **2828** (Bethlehem): 6 miles [9.65 km] southeast of Bethlehem on road to Clarens (-AB), 13 February 1967, *Scheepers 1374* (PRE); Spitskrans Plaas (-AD), 22 September 1976, *Muller 1926* (PRE); Bethlehem, Qwaqwa Nasionale Park (-BC), 23 November 1994, *Zietsman 2539* (NH). **2829** (Harrismith): *voet van* [at the foot of] Platbergaansuidekant (-AA), 14 November 1973, *Muller 1254* (PRE);

Harrismith District, Kings Hill Botanical Garden (-AC), 02 November 1966, *Rycroft 2992* (NBG); Near Sterkfontein Dam (-AC), 10 November 1972, *Van der Zeyde s.n.* (NBG); Sterkfontein Dam (-AC), 16 October 1974, *Jacobsz 1834* (PRE); Drakensberg Botanic Garden (-AC), 08 February 1978, *Jacobsz 2183* (NBG); Harrismith District, Middelspruit (-AC), 15 January 1920, *Putterille s.n.* (BOL; PRE); Platberg near point where One Man's Pass starts (-AC), 14 November 1983, *Roux 1618* (PRE). **2926** (Bloemfontein): Thaba Nchu Berg (-BB), 29 October 1991, *Engelbrecht JE 600* (PRE). **3027** (Lady Gray): Kranskop, Zastron (-AC), 08 November 1973, *Ferreira 84* (PRE). **KWAZULU-NATAL PROVINCE**: **2729** (Volksrust): Just outside Newcastle on road to Memel (-DB), 24 October 1982, *Germishuizen 2409* (PRE). **2730** (Vryheid): Utrecht (-CB), October 1927, *Thode A1286* (PRE). **2731** (Louwsburg): 11 km from Kongolwane on road to Louwsburg (-CA), 18 October 1982, *Germishuizen 2196* (PRE); 12 km from Louwsburg on road to Nongoma (-CB), 17 October 1982, *Schrire 1102* (NH); Louwsburg District: 21 km from Louwsburg on road to Ngome Forest (-CB), 17 October 1982, *Germishuizen 2155* (PRE); Near Gluckstadt (-CC), 09 October 1969, *Strey 9110* (PRE). **2828** (Bethlehem): Royal Natal National Park, Vemvaan River Valley near junction with Tugela (-DB), 07 February 1982, *Hilliard & Burtt 15464* (NU; PRE). **2830** (Dundee): Dundee district, 2 km *vanaf* [from] Pomeroy *op pad na* [on road to] Dundee (-CB), 12 December 1973, *Grobbelaar 1782* (PRE); 5 km from Kranskop to Eshowe (-DD), 11 October 1975, *Stirton 5207* (PRE). **2831** (Nkandla): Babanongo District, near Denny Dalton (-AC), 16 January 1946, *Acocks 12313* (PRE). **2929** (Underberg): Giants Castle Game Reserve (-AB), 10 March 1961, *Skead 90* (NU); Willowgrange (-BB), 08 October 1975, *Stirton 5109* (PRE); Mkifa Valley, Glengariff farm (-DC), 24 February 1990, *Stirton 12714* (NU); Ixopo, 6 miles [9.65 km]

Eastwolds on Hella Hella-Richmond road (-DD); 27 March 1963, *Edwards 3114* (NU; PRE); Impendle (-DD), 31 May 1979, *Phelan 383* (NU). **2930** (Pietermaritzburg): Howick, 2 km from N3 fly-off (-AC), 16 November 1988*, Edwards 381* (NU; PRE); *Edwards 383* (NU); Just outside Greytown, Cooper Street Extension (-BA), 11 November 1983, *Schrire 1560* (NH); Greytown (-BA), December 1932, *Wylie s.n.* (NH); Greytown District, 2-3 km outside town on road to Stanger (-BA), 13 October 2001, *Meyer 3927* (PRE); 20 km to Kranskop from Greytown (-BB), 13 September 1974, *Stirton 1049* (PRE); 18 miles [28.96 km] from Pietermaritzburg on Greytown Road (-CB), (Precise date not provided), *Wells 4019* (PRE); Richmond road to Pietermaritzburg (-CB), 11 January 1947, *Barker 4386* (BOL; NBG); Maritzburg, Alexandra Park (-CB), 15 October 1939, *Fairall 86* (BOL); 2-3 miles [±4.8 km] along road to Manderston via Bistey (-CB), November 1962, *Morris 86* (NU); Scottsville (-CB), May 1946, *Curry 8* (NU); Zwartkop (-CB), 30 September 1964, *Moll 1129* (NU); Camperdown, Cato Ridge (-DA), 14 January 1962, *Keytel 49* (PRE); January 1941, *Macpherson 37* (NU); Weza (-DA), 25 February 1978, *Coleman 1007* (NH). **3029** (Kokstad): Bedford, Harding (-DB), 29 October 1963, *Lennox s.n.* (NU). **3030** (Port Shepstone): Ixopo District, Craighton (-AA), 05 November 1964, *Shirley s.n.* (NU); Oribi Aerodrome (-CA), 24 September 1965, *Moll 2365* (PRE). **WESTERN CAPE PROVINCE**: **3420** (Bredasdorp): Overberg District, De Hoop, Witwater (-AD), 06 August 1984, *Fellingham 693* (PRE). **EASTERN CAPE PROVINCE**: **3026** (Aliwal North): Eland's Hoek near Aliwal North (-DA), October (No year)*, Bolus 137* (BOL). **3027** (Lady Grey): Zastron District, Aasvoelberg by Zastron (-AC), 18 December 1991, *Peyper 1190* (PRE). **3028** (Matatiele): E. facing slope above forest patch across river from York (-AD); 14 October 1987, *Strever 332* (PRE). **3029** (Kokstad): Griqualand East, on grassy slopes

near Clydesdale (-BD), November 1884, *Tyson 1055* (BOL; NBG; PRE); Griqualand East (-BD*),* March 1885, *Tyson 2065* (NBG); Mvenyane, Cedarville (-CA), 18 November 1920, *Bandert 89* (GRA); Ngeli, Mpetsheni (-DD), February 1983, *Gettiffe 1211* (NU). **3126** (Queenstown): Sterkstroom, Penhoek Pass (-BC), 28 December 1962, *Barker 9841* (BOL; NBG); 19 km from Dordrecht on road to Queenstown (-DB), 13 January 1997, *Germishuizen 8914* (PRE); Queenstown: Upper region (-DD), February 1920, *Page 16979* (BOL). **3127** (Lady Frere): Dordrecht, 2 km out of town on way to Barkly East (-AC), 28 January 1992, *Le Roux 1952* (GRA); 5 km from Indwe on road to Elliot (-AD), 11 January 1997, Germishuizen 8771 (GRA, PRE); Indwe to Lady Frere, Route 396 (-CB); 11 January 1997, *Bredenkamp 1092* (PRE). **3128** (Mthatha): Tsolo Agricultural College, north eastern base of Isitsila Hill (-BD), 25 November 1991, *Cloete 1375* (NH); Mthatha (-DD), 25 January 1983, *Germishuizen 2414* (PRE); Shawbury 3 km *van af brug oor* [from the bridge over] Tsitsa Rivier (-BB), 07 October 1982*, Van Wyk & Kok 5817* (PRE). **3225** (Somerset East): Somerset Oos, Bosberg (-DA), 26 November 1967, *Van der Walt 226* (PRE); near Somerset East (-DA), 06 March 1866, *Bolus 2049* (BOL). **3226** (Fort Beaufort): About 5 km above Nico Malan Pass (-BD), 07 February 1995*, Victor & Hoare 331* (PRE); Amatola Mountains, road between Cathcart and Hogsback (-BD), 27 January 1995, *Victor & Hoare 550* (PRE); Katberg District, Maasdorp Valley, Stockenstrom (-DA), October 1932, *Hollard 3971* (BOL); Katberg (-DA), January 1894, *Smith s.n*. (NU); Mpofu Game Reserve, high plateau near gate to Retief Post (-DA), 28 February 2006, *Von Staden 28* (PRE); University of Fort Hare, Hillsides above Fort Hare Weir (-DD), 29 December 1942, *Giffen 1370* (GRA;NU;PRE). **3227** (Stutterheim): Cathcart District, Thomas River (-AD), 15 January 1947, *Compton 19310* (NBG); Thomas River (-AD), 15

January 1947, *Leighton 2798* (BOL); Dohne, Fort Cunynghame (-AD), (Precise date not provided), 1890, *Sim 19457* (NU; PRE); 52 km from Engcobo on road to Cofimvaba (-BA), 12 January 1997, *Germishuizen 8868* (PRE); Komgha, Junction Farm (-DB), 26 November 1945,*Compton 17757* (NBG); Stutterheim (-CB), December 1913, *F.A. Rogers 12740* (BOL; PRE); St John's Dohne No.690 (-CB), 06 December 1942, *Acocks 9415* (PRE); Dohne Hill (-CB), May 1892, *Sim 1266* (NU); Victoria East: Black Hill, Lovedale (-CB), 09 December 1943, *Barker 2753* (BOL; NBG); King William's Town, near Kei Road village (-CD), 21 February 1960, *Ranger 230* (PRE); King William's Town (-CD), January 1891, *Sim 1268* (NU); Cathcart (-DA), 01 February 1982, *Le Roux 82/02* (GRA); Komgha, grassland ca. 5 km W. of Town (-DB), 01 November 1987, *Goldblatt & Manning 8545* (NBG; NU); Near Komgha (-DB), December 1890, *Flanagan 764* (PRE). **3326** (Makhanda): Turnoff to Alexandria Forest (-CB), 08 March 1954, *Johnson 870* (GRA; PRE)**. 3327** (Peddie): East London (-BB), 25 December 1905, *Galpin TRV 4606* (PRE); East London District, Keiskama, edge of Lagoon (-AA), December 1902, *Rattray 7698* (PRE).

LESOTHO: **3027** (Matatiele): Mohale's Hoek, Sekake's (-AB), 16 January 1969, *Jacot Guillarmod 6450* (GRA). **3028** (Matatiele): Hilltop Ha Mokife, between Qacha's Nek and Ramatseliso's Gate (-BB), November 1979, *Schmitz 8821* (PRE).

3.3.2. ***Rhynchosia albissima*** Gand. in Bull. Soc., Bot. France 60: 461 (1913); Baker f., Legum. Trop. Africa: 487 (1929); Verdcourt in F.T.E.A., Leguminosae, Pap. : 752 (1971); Drummond in Kirkia 8: 225 (1972); Thulin in Fl. Somalia 1: 445 (1993); Lock, Leg. Afri. Checklist: 424 (1989).

=*Rhynchosia albomarginata* Chiov., Fl. Somalia 2: 170 (1932). Type: Somalia, *Oltregiuba, Colbio pianta erbaea VII fi*, 1931, *Tozzi 259* (FT!, holotype).

=*Rhynchosia memnonia* sensu Harvey in F.C. 2: 253 (1862) pro parte, non (Del.) DC.

Perennial, robust, erect subshrub or woody herb, 0.3–3 m high. *Stems* pubescent, the younger branches densely pubescent with silvery velvety or grey hairs. *Leaflets* 14–42×10–30 mm, paler beneath, usually elliptic to obovate, round or rhombic, seldom acute, densely pubescent to velvety with silver-grey hairs on both sides; venation prominent on the lower surface; petiole 10–35 mm long; stipules 2–6×1–2 mm, pubescent, ovate-lanceolate. *Inflorescences* in axillary or terminal racemes, dense, 30–170 mm long, pubescent. *Flowers* 6–10 mm long, yellow with brown or maroon venation on petals, pubescent. *Fruits* 12–22×4–6 mm, oblong-falcate, covered with silvery-grey hairs. Flowering time: from early spring through to early winter (August–May).

Diagnostic characters: *Rhynchosia albissima* closely resembles *R. minima.* var. *memnonia*, with its leaves silky-silvery to velvety or cinereous pubescent on both surfaces, but it can be distinguished by growth form; *R. albissima* has a rigid stem and is usually a woody erect herb, growing up to 2 m high, whereas *R. memnonia* is a prostrate herb, with twining or trailing stems.

Distribution and habitat: *Rhynchosia albissima* is widespread from northeast South Africa, eSwatini into Mozambique and Zimbabwe northwards to East African, including Uganda, Tanzania, Kenya and Somalia (Verdcourt, 2001). In South Africa, it occurs in Limpopo, North West, Gauteng, Mpumalanga and KwaZulu-Natal Provinces (Figure 3.2). It prefers warm to hot low rainfall areas, floodplain alluvium and black clay soils, pan

margins, scrub mopane on stony ground, and also in cultivated fields; at an altitude of 40–500 m.a.s.l. (Verdcourt, 2001).

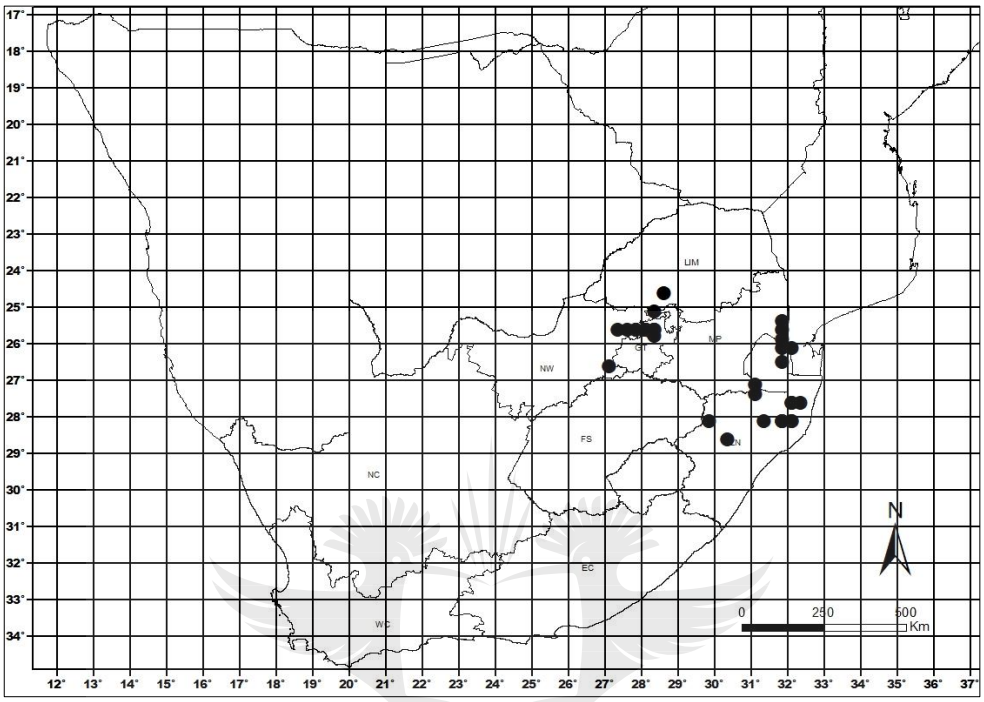

Figure 3.2: Known geographic distribution of *Rhynchosia albissima* in southern Africa

Conservation status: LC (Raimondo et al., 2009)

Additional specimens examined

SOUTH AFRICA, **LIMPOPO PROVINCE**: **2428** (Modimolle): Mookgophong (Naboomspruit), Mosdene (-DA), 26 November 1919, *Galpin 98M* (PRE). **NORTH WEST PROVINCE**: **2527** (Rustenburg): Zwartruggens (-DA), 18 December 1949, *Sutton 1269* (PRE); Between Magaliesburg and Rustenburg, 12 April 1954, *Schlotfeldt s.n.* (NBG); Pretoria District, Hartebeestpoort (-DB), 26 December 1919, *Findlay 21* (PRE); Bokfontein (-DB), November 1909, *Jenkins TRV 7535* (PRE); **2627** (Potchefstroom):

Noyjons (-CA), 20 January 1977, *Dannhauser 7* (PRE). **GAUTENG PROVINCE**: **2528** (Pretoria): East of Pienaars River on road to Rust de Winter (-AB), 14 October 1996, *Germishuizen 8686* (PRE); Rooikop (-AB), 13 December 1932, *Smuts and Gillett 2105* (PRE); Willow Glen (-CA), 30 November 1959, *Strey SKF878* (PRE); Below the Wonderboom-in camp between the Poplar Grove and the Dipping Tank (-CA), March 1932, *Smith 6237* (PRE); Near Onderstepoort, above right bank of Aapies River at the railway bridge (-CA), 15 February 1932, *Smith 6049* (PRE); Onderstepoort Nature Reserve (-CA), 17 May 2018, *Mothogoane & Masupa 21* (PRE); Wonderboompoort (-CA), 29 November 1904, *Leendertz 440* (NH;PRE); Villeria (-CA), 20 December 1919, *Mogg 7325* (PRE); Wonderboom Reserve, lower South slopes (-CA), 09 November 1945, *Repton 2761* (PRE); Wonderboom, grassy plot between the two furrows below old dipping tank (-CA), 24 January 1926, *Smith 2240* (PRE); Wonderboompoort (-CA), 22 December 1904, *Leendertz 484* (BOL; PRE); Gezina (-CA), 10 March 1914, *Mogg 10082* (BOL; PRE); Doornpoort, new extension in Bougainvilla road close to Zambesi Estate Development (-CB), 03 December 2005, *Bester 6065* (PRE); Willows open plot (-CB), 08 November 1977, *Germishuizen 467* (PRE); 22 km from Pretoria on road to Rayton (-CB), 13 December 1980, *Stirton 8481* (PRE); Six fountains site along edge of natural donga (-CD), 26 October 2005, *Lemmer 607* (PRE). **2531** (Komatipoort): Kruger National Park, *Suid-oostelike gebied, groei binne* 20 m *van paaie af* [South-eastern area, growing within 20m of roads] (-BD), 21 April 1987, *Wentzel N.110* (PRE); Kruger National Park, Lebombo bridge (-DB), 09 November 1954, *Van der Schijff 3979* (PRE); 36 km from Komatipoort road to Stegi (-DD), 08 March 1979, *Balsinhas 3396* (PRE). **KWAZULU-NATAL PROVINCE**: **2732** (Ubombo): 21 km from Josini on road to Ingwavuma (-AA),

28 November 1978, *Germishuizen 1014* (PRE); 1 mile [1.60 km] SW of Jozini Dam (-AC), 19 November 1967, *Strey & Moll 3634* (PRE); Umkuzi Station (-CA), 08 November 1944, *Gerstner 4947* (PRE); 3 km south of turnoff to Mkuze G.R. on untarred road to Hluhluwe (-CA), 21 February 1982, *Reid 509* (PRE); Mkuzi, slopes of Lebombo Mountain (-CB), 27 January 1932, *Galpin 133121* (BOL; PRE); Mkuzi Game Reserve Mshopi (-CB), 29 February 1976, *Goodman 530* (NU); 36 km from Mkuze to Mtubatuba (-CC), 12 May 1981, *Stirton 8840* (NH; PRE); Ubombo, turnoff to Sodwana Bay from N2 North of Hluhluwe, in mown grass on side of highway (-CC), 12 May 1981, *Schrire 358* (NH); Near River Umsinduzi (-CC), 18 April 1888, *Wood 820* (BOL); Ubombo Coastal Belt (-CC), 18 January 1949, *Comins s.n.* (NU). **2829** (Harrismith): Hluhluwe Game Reserve, Amanzimnyama Catchment (-BB), 01 November 1965, Bourquin and Fakude 14 (PRE). **2830** (Dundee): 6 m [9.65 km] N. of Tugela Ferry (-CB), 14 January 1951, *Codd 6332* (PRE). **2831** (Nkandla): 5 miles [8.04 km] N. of Mahlabathini (-AB), 14 January 1948, *Acocks 13970* (PRE); Hlabisa, Hluhluwe Game Reserve (-BB), 10 November 1958, *Guy 29* (NU; PRE); near entrance to Hluhluwe Game Reserve from Hluhluwe (-BB), 15 October 1946, *Codd 2041* (PRE); Near Hluhluwe Station (-BB), 15 October 1946, *Acocks 13104* (PRE). **2832** (Mtubatuba): Hluhluwe Game Reserve (-AA), 11 January 1956, *Ward 2957* (NU; PRE); Hluhluwe Game Reserve (-AA), 07 July 1951, *Johnson 53* (BOL; NBG). **2930** (Pietermaritzburg): Makatini Flats (-CB), February 1975, *Van Rensburg 27* (NU).

ESWATINI: **2631** (Mbabane): Siteki District, Nokwane (-BB), 13 October 1961, *Compton 30840* (PRE); May 1910, *Stewart TRV 10360* (PRE). **2632** (Bella Vista): Mbuluzi Private Nature Reserve 1 mile [1.6 km] E. of Maphiveni, old compound known as Mbilinga's (-AA), 20 February 1980, *Culverwell 1349* (PRE).

3.3.3. ***Rhynchosia caribaea*** (Jacq.) DC., Prodr. 2:384 (1825); E.G. Baker, Legum. Trop. Africa: 475 (1929) pro parte; Meikle in Kew Bull. 6: 173, fig. 1 (1951); Mogg in Macnae & Kalk, Nat. Hist. Inhaca Isl., Mozamb., ed. 2. rev.:146 (1969); Drummond in Kirkia 8:225 (1972); Goncalves in Garcia de Orta, Sér. Bot. 5: 106 (1982); Lock, Leg. Afr. Checklist: 426 (1989). *Glycine caribaea* Jacq., Collect. 1: 66 (1787); Icon. Pl. Rar. 1: t.146 (1787).

=*Rhynchosia gibba* E. Mey. in Linnaea 7: 170 (1832); Harvey in F.C. 2:255 (1862). *Copisma gibbum* (E. Mey.) E. Mey., Comment. Pl. Afr. Austr.: 137 (1836).

=*Rhynchosia acuminata* Eckl. & Zeyh., Enum. Pl. Afr.: 253 (1836).

=*Rhynchosia flavissima* sensu Baker f. in J. Linn. Soc., Bot. 40:59 (1911), non Hochst.ex Baker.

=*Rhynchosia gazensis* Baker f., Legum.Trop. Africa: 475 (1929). Type: Zimbabwe, Gazaland, Chirinda, *Swynnerton* 448 (K!, holotype).

Perennial, herbaceous climber or creeper, growing from a thick woody rootstock, 0.3–1.0 m long. *Stems* slender, twining or procumbent, pubescent. *Leaves* heart-shaped, terminal leaflets10–60×8–55 mm, broadly rhombic or triangular, gradually narrowed above to an obtuse or sub-acute apex. Lateral leaflets obliquely rhombic-ovate with lower margin distinctly convex, pubescent or glabrescent, some densely velvety, gland dotted; petiole 0.5–7.5 cm long; stipules at right angles to stem, 2–7×0.5–3.0 mm, ovate to lanceolate, acuminate. *Inflorescence* lax, in axillary racemes, slender, viscid, 40–230 mm long. *Flowers* 9–20 mm, yellow with purple or dark blue stripes, glabrous. Fruits 20–35×5–9 mm, falcate, sub-inflated, and pubescent, and with sparse to dense long silky glandular (golden) hairs. Flowering time: from mid-spring through to mid-summer (October–January).

Diagnostic characters: *Rhynchosia caribaea* is often confused with *R*. *minima* (L.) DC. var. *minima* because of its heart-shaped leaves, but it can be distinguished from *R. minima* by its larger flowers (9–20 mm long in length versus up to 8 mm long in length). *R. caribaea* has a sub-inflated, larger fruit (20–35×5–9 mm) when compared to the compressed smaller fruit (6–22 × 3–6(7) of *R. minima.*

Distribution and habitat: *Rhynchosia caribaea* is widely distributed in southern Africa, including Namibia, Botswana, South Africa and eSwatini. It is also found in Zimbabwe, Mozambique and Congo (Verdcourt, 2001). In South Africa, it occurs in Limpopo, North West, Gauteng, Mpumalanga, Free State, KwaZulu-Natal, Western Cape and Eastern Cape Provinces (Figure 3.3). It grows in grassland, forest margins; scrub in gully, wooded hillsides, near streams and on seaward side of dunes.

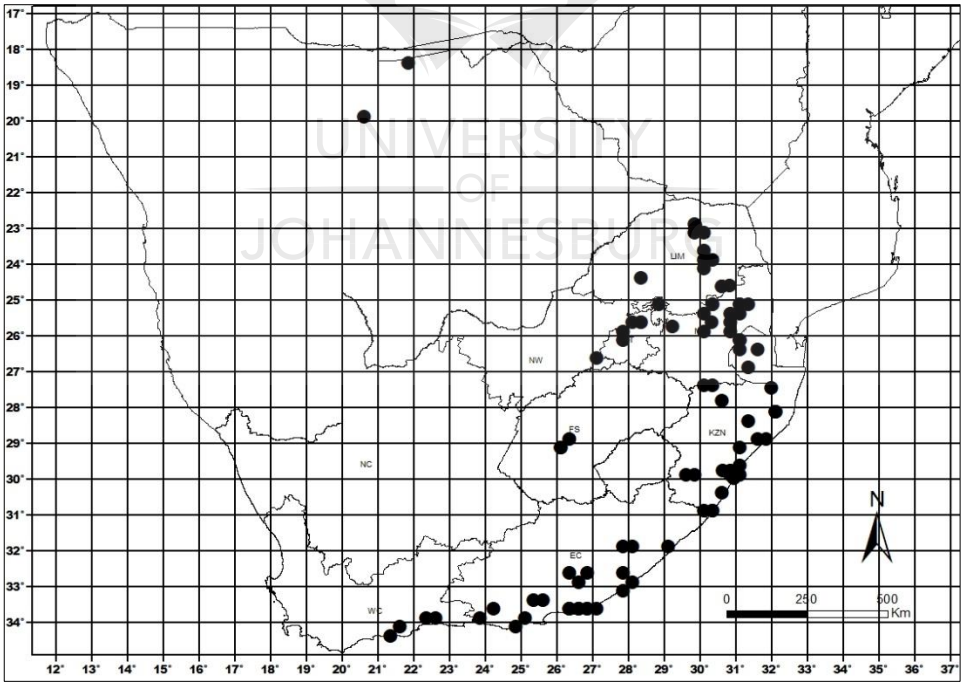

Figure 3.3: Known geographic distribution of *Rhynchosia caribaea* in southern Africa

Conservation Status: LC (Raimondo et al., 2009)

Additional specimens examined

NAMIBIA: 1920 (Tsumkwe): Grootfontein District, 20 miles [32.18 km] SW of Nama Pan (-DC), 16 August 1955, *Story 5222* (PRE).

BOTSWANA: 1821(Northern District): Samocima Lediba (-BD), 20 February 1979, *Smith 2705* (PRE).

SOUTH AFRICA, LIMPOPO PROVINCE: 2229 (Polokwane): Northern side of Soutpansberg, Farm Little Leigh (-**DD**), 27 March 2003, *Jordaan 4125* (PRE); Louis Trichardt District: ±13 km N. of Louis Trichardt on Little Leigh Farm (-**DD**), 03 April 1997, *Burgoyne 6058* (PRE); Zoutpansberg Mountains about 5 miles [8.04 km] above Louis Trichardt (-DD), 06 March 1948, *Rodin 3984* (BOL). **2330** (Tzaneen): Gazankulu, Giyani District, Chavani (-AA), 01 May 1977, *Liengme 74* (PRE); Westfalia Estate, near Duiwelskloof, N. Transvaal, Rosenthal scrub forest, behind Macs Avocado-grove (-CA), 15 February 1964, *Bos 1334* (PRE); De Hoek Forest Station, Magoebaskloof (-CC), 31 March 1972, *Clarke 375* (PRE); Giyani, Farm Janetsi on road to Letsitele (-CD), 13 June 1996, *Jacobsen 5320* (PRE). **2428** (Modimolle): Waterberg District, Geelhoutkop on farm of Van Staden (-AD), 11 March 1978, *Germishuizen 774* (PRE). **2430** (Pilgrim's Rest): Lekgalameetse Nature Reserve, The Downs, near The Downs-house (-AA), 18 March 1986, *Stalmans 1172* (PRE); Makoetsikloof, Trichardtsdal (-AB), 14 June 1975, *Hall 4565* (NBG). **NORTH WEST PROVINCE**: **2527** (Rustenburg): Near Rustenburg, on Zeerust road (-CA), 06 February 1929, *Hutchinson 2935* (BOL). Sandspruit (-CB), 14 February 1946, *Acocks 12438* (PRE); Brits District, South of Hartebeespoort Dam, Farm Haddedah

Boerdery, along Hennops River (-DD), 22 February 1994, *Heymann, Cloete & Burgoyne 49* (PRE); Schoemansville Valley (-DD), 28 October 1934, *Esterhuysen 25* (BOL). **2627** (Potchefstroom): Mountain Kloof (-**CA**), 02 October 1944, *Louw 1021* (PRE). **GAUTENG PROVINCE**: **2528** (Pretoria): Kwa-Ndebele, farm Boekenhoutfontein (-BB), 17 March 1981, *Van Hoepen 1645* (PRE); Fountains Valley (-CA); 25 March 1929, *Verdoorn 780* (PRE); 22 February 1929, *Verdoorn 742* (NH; PRE); Roodeplaatdam Natuur Reservaat (-CB), 21 September 1979, *Van Rooyen 1925* (PRE); Roodeplaat Dam Nat. Res. P.1. KJV (-CB), 16 November 1979, *Van Rooyen 2198* (PRE); Cullina, Muningi Gorge, along the river bank hiking trail (-DA), 04 May 2018, *Mothogoane & Sepheka 12* (PRE). **2627** (Potchefstroom): Roodepoort, Walter Sisulu National Botanical Garden, Roodepoort (-BB), 16 February 1983, *Behr 410* (NBG). **MPUMALANGA PROVINCE: 2430** (Pilgrim's Rest): Sekhukhuneland District, Abel Erasmus Pass, near top of Pass on slopes above rest site (-DA), 06 April 1994, *Burgoyne 2410* (PRE); Lydenburg District, Kaspersnek, Foot of the nek on Ohrigstad road (-DA), 23 January 1968, *Hilliard 4722* (NU; PRE); Mariepskop, rainforest near Blyde turnoff (-DB), 31 March 1959, *Van der Schijff 4592* (PRE). **2529** (eMalahleni): Middelburg District, BuffelsVlei (-CA), 23 November 1934, *Rudatis 58* (PRE). **2530** (Lydenburg): 4 km along Boschhoek road on Lydenburg/ Dullstroom road (-AB), 13 March 1989, *Germishuizen 5187* (PRE); Lydenburg Farm Kwaggashoek 66 JT, at waterfall (-AB), 12 February 1986, *Krynauw 1091* (PRE); 5 km from turnoff of Vermont and Rooikrans on road to Oshoek (-AC), 05 March 1979, *Germishuizen 1067* (PRE); 10 km from Lowveld Botanic Garden on road to Witrivier (-BD), 23 November 1993, *Germishuizen 6982* (PRE); Waterval-Boven, picnic spot at the entrance of tunnel (-CB), 10 May 1995, *Jordaan 2886* (PRE); 21 km from Machadodorp

on road to Badplaas (-CC), 08 March 1989, *Germishuizen 5020* (PRE); Kaapsche Hoop (-DB), March 1918, *Rogers 20781* (PRE); Nelshoogte Mountain Pass, 31 km from Barberton on road to Badplaas (-DD), 05 March 1986, *Germishuizen 3837* (PRE). **MPUMALANGA PROVINCE**: **2530** (Lydenburg): Dullstroom, amongst dolerite rocks at base of Elandskop, near Maak siding (-CC), 26 January 1933, *Galpin s.n*. (BOL). **2531** (Komatipoort): Hazyview, 1 km outside of town (-AA), 21 February 1977, *Germishuizen 150* (PRE); Kruger National Park (-AB), 22 February 1949, *Codd 5190* (PRE); 9 km from Kanyamazane turn-off on road to Mbombela (-AC), 08 March 1989*, Germishuizen 5158* (PRE). **2730** (Vryheid): Wakkerstroom, Town lands (-AC), February 1917, *Beeton PRE 61275* (PRE); Wakkerstroom District, Plaas Retirement, 20 km *van af* [from] Wakkerstroom *op* [on] Piet Retief *pad* [road] (-AD), 24 October 1994, *Meyer 209* (PRE). **FREE STATE PROVINCE**: **2826** (Brandfort): Bloemfontein District, Glen Landboukollege (-CD), 21 March 1985, *Zietsman & Zietsman 356* (PRE). **2828** (Bethlehem): Witzieshoek (-DB), March 1906, *Thode 5690* (NH). **2926** (Bloemfontein): Bayswater farm (-AA), 21 March 1925*, Potts BLF 3858* (PRE); Winter Valley (-AA), 11 March 1967, *Muller PRE 56069* (PRE); Free State National Botanical Garden (-AA), 28 March 1973, *Kriel s.n.* (NBG); Orange Free State Botanical Garden near Bloemfontein (–AA), 11 March 1967, *Muller 65* (NBG). **KWAZULU-NATAL PROVINCE: 2632** (Bela Vista): Ingwavuma District, Kosi Estuary, near school on ridge (-DD); 18 November 1982, *Balkwill 547* (NU). **2730** (Vryheid): Amajuba District, Utrecht Municipality Area, Luiperdkloof Farm, Natural Heritage Site No. 479 (-DC), 25 January 2011, *Mnxati 305* (NH). **2732** (Ubombo): 13 miles [20.92] E. of Pongola River to Maputa (-AB), 20 November 1969, *Moll 4626* (NH; PRE); Jozini Pass (-AC), 11 March 2004, *Bester 4891* (PRE); ca. 2 km from turnoff to

Phelindaba on Mbazwana road (-BA), 3 December 1985, *Germishuizen 3591* (PRE). **2829** (Harrismith): Oliviershoek Pass (-CA), 13 April 1963, *Ross 150* (NU); Bergville, Umlambonja Valley, Cathedral Peak area, Drakensberg (-CB), 09 February 1954, *Schelpe 77* (BOL). **2830** (Dundee): Tugela Ferry (-CB), 05 February 1939, *Galpin s.n*. (BOL). **2831** (Nkandla)**:** Empangeni District**,** 17 km from Empangeni to Richards Bay (-AD), 11 May 1981, *Stirton 8809* (PRE); 20 km from Eshowe to Gingindhlovu (-CA), 10 May 1981, *Stirton 8767* (PRE); Eshowe District, Ngoye Forest (-DC), 28 March 1907, *Wylie & Wood 10333* (NU; PRE); 8 km from Empangeni to Richards Bay (-DD), 11 May 1981, *Stirton 8805* (NH); 43 km NE of Empangeni on road to Hluhluwe (-DD), 12 August 1970, *Brummit 12493* (PRE); Mtunzini District: Tugela mouth (-DD), 09 February 1964, *Hilliard 2735* (NU). **2832** (Mtubatuba): Hlabisa District, Hluhluwe Game Reserve (-AA), 16 October 1946, *Acocks 13165* (PRE); *Codd 2045* (PRE); 24 August 1962, *Fakude 9* (NU; PRE); Corridor, South side of Msimba Hill (-AA), 05 April 1955, *Player 65* (NU; PRE) St. Lucia Estuary Game Park (-AD), 10 April 1978, *Pooley 2024* (NU). **2929** (Underberg): Nkonzo State Forest, Kwa-yili Forest (-DC), 20 February 1984, *Nicholas & Van der Berg 1836* (NH; NU); Bulwer, Sunset Farm (-DC), 24 February 1990, *Vos 48* (NU); Midlands, Bulwer/Hlanganani District, E Chibini Village, ca. 700 m from eChibini/Bulwer Town road (-DD), 27 February 2001, *Ngwenya 2124* (NH). **2930** (Pietermaritzburg): Karkloof (-AC), 24 March 1898, *Wood 7209* (BOL); Kunhardt's farm, right hand turnoff 21 km from Merrivale on Boston road (-CA), 25 April 1982, *Kunhardt 84* (NU); Bisley (-CB), 07 April 1961, *Ross s.n.* (NU); Camperdown District, Shongweni Hillside, above Shongweni Dam (-DC), 06 September 1964*, Ross 1285* (NH; NU); Greater Durban Metropolitan Area, Hammarsdale area, Hector-ESKOM substation site (-DC), 13 April 1995, *Ward 13004*

(NU; PRE); Wentworth Bluff, Durban District (-DD), 08 March 1967, *Ward 6135* (NU; PRE); Isipingo North (-DD), 12 March 1967, *Ward 6172* (NU;PRE); Isipingo North (-DD), 30 March 1967, *Ward 6214* (NU; PRE); Greater Durban Metropolitan Area, Kloof Extension 13, Phase 1 (-DD), 16 April 1995*, Ward 13045* (NH; NU); Umbogintwini River, AECI Effluent Dams, west side of Dam 1 (-DD), 15 May 2006, *Lubke & Vronons 5450* (GRA). **2931** (Stanger): Mapumulo District, Umshaba Falls, Oqaqeni (-AA), 29 March 1957, *Edwards 1814* (NU; PRE); Melmoth/Eshowe turnoff on N2 (-BA), 10 May 1981, *Schrire 327* (PRE); Inanda District, 5 miles [8.04 km] W. of Verulam (-CA), 16 September 1966, *Moll 2077* (NU); Treasure Beach (-CC), 14 February 1985, *Ngwenya 115* (PRE); Durban District, Fynnlands (-CC), April 1945, *De Villiers 27* (NU). **3029** (Kokstad): Ngele, Bangani Forest (-DA), 27 January 1990, *Abbott 5101* (NH). **3030** (Port Shepstone): Umzinto District, Vernon Crookes Nature Reserve (-BC), 02 October 1983, *Balkwill & J. Manning 1024* (NU); Park Rynie, near railway line (-BC), July 1948, *Nixon 25* (NU); Pennington, South Coast (-BC), April 1950, *Gower 19* (NU); Umzinto District, Park Rynie Beach, N of Park Rynie Station (-BC), 08 April 1967, *Baijnath 378* (NU; PRE); Oribi Gorge (-CA), 23 February 1963, *Hilliard 2781* (NU). Marburg (-CB), April 1986, *Edwards 158* (NU); Ramsgate (-CC), 09 May 1982, *Schrire 920* (PRE); Palm Beach (-CD), 25 October 1975, *Stirton 5670* (PRE); Glenmore Beach, about 15 km North of Port Edward on road to Margate, near sea (-CD), 18 May 1996, *Germishuizen 8657* (PRE); Ramsgate Beach, South Coast (-CD), 01 July 1984, *Vos & Gormley 307* (NU). **NORTHERN CAPE PROVINCE**: **2821** (Upington): Currie, Griqualand Orientalis (-CA), precise date not provided, 1883, *Tyson 1696* (BOL). **WESTERN CAPE PROVINCE**: **3322** (Oudtshoorn): George District, Wilderness (-DC), 14 November 1952, *Van Niekerk 193* (BOL; PRE); 17

August 1944, *Compton 15764* (NBG); Kaymans River (-DC), April 1928, *Fourcade 3884* (BOL). **3421** (Riversdale): Stillbay west end of Platbos (-AD), 24 April 1979, *Bohnen 5583* (PRE); 0.5 miles [0.80 km] along road to Couritz River mouth, 5 miles [8.04 km] East of Albertina (-BA), 01 August 1972, *Taylor 8143* (PRE). **EASTERN CAPE PROVINCE**: **3029** (Kokstad): 1 km from Fort Donald Store to Bizana (-DC), 21 January 1986, *Van Wyk 7141* (NH). **3128** (Mthatha): Elliotdale District, The Haven (-DC), 15 November 1966, *Gordon-Gray 1181* (NU). **3129** (Port St. John): Ngqeleni District, Mthatha River mouth (-CC), 12 April 1947, *Acocks 13570* (PRE). **3130** (Port Edward): Mzamba River Crossing on the way to Mnyameni (-AA), 20 March 1987, *Van Wyk 8071* (GRA; NH). **3226** (Fort Beaufort): Fort Fordyce (-CB), 12 March 1947, *Story 2118* (PRE); Braeside, 10 miles [16.09 km] N. of Fort Beaufort (-DC), 06 April 1947, *Story 2222* (PRE). **3227** (Sutterheim): Near Komgha (-DB), April 1891, *Flanagan 719* (PRE). **3228** (Butterworth): Seashore, HagaHaga (-CC), 05 April 1973, *Clarke 487* (PRE); East London, Gonubie mouth (-CC), 04 October 1942, *Acocks 9148* (PRE); Kentani, Nxoxa mouth (-DA), 12 July 1966, *Strey 6692* (NU; PRE). **3324** (Steytlerville): Baviaanskloof, opening in forest in Geelhoutboskloof (**-**CB), 20 April 2012, *Euston-Brown 1947* (NBG); Uniondale: Klein River, along road from Kouga River (-DD), October 1933, *Fourcade 5098* (NBG). **3325** (Port Elizabeth): 8 miles [12.87 km] from Zuurberg Inn on Addo Road (-DA), 11 March 1970, *Wells 4208* (PRE); Zuurberg National Park (-BC), 09 January 1986, *Van Wyk & Van Wyk 1383* (PRE); Port Elizabeth, Van Staden Rivier Pass (-CC), 18 June 1978, *Grobbelaar 2459* (PRE); Port Elizabeth (-DC), 07 November 2000, *Burrows 5555* (GRA). Patensie Hills (-DD), 13 April 1952, *Barker 7900* (NBG); Kabeljous River near drift (-DD), November 1921, *Fourcade 1757* (BOL). **3326** (Makhanda): Fish River, Dohle Drift Camping Site (-BB), 06 August 2006,

Dold 4552 (GRA); Albany, Makhanda (Grahamstown) (-BC), (Precise date not provided), *Rogers 28347* (BOL); East Griqualand, 08 March 1965, *Nicholson 271* (NH); Featherstone Kloof (-BC), 12 July 2001, *Kayombo 3647* (GRA); De Mond, Alexandria (-CB),12 March 1970, *Wells 4220* (PRE); 10 miles on Alexandria Road from Johannesburg and Port Elizabeth intersection (-CB), 12 March 1970, *Wells 4216* (PRE); Alexandria, Woody Cape-Alexandria Coast (-CD), 23 September 1994, *Dold 976* (GRA); Bushman's River mouth (-DA), 12 April 1954, *Johnson 897* (PRE); Bushman's River mouth (-DA), 12 May 1931, *Galpin 10636* (BOL;PRE); Kenton-on-Sea, near mouth of Bushmans River (-DA), 08 May 1997, *Burrows 4617* (GRA); Makhanda, along main road between Port Alfred and Fish River mouth (-DB), March 1978, *Gibbs Russell & Robinson 4420* (BOL; PRE); Port Alfred (-DB), April 1916. *Tyson 12620* (PRE); Amatole Mountains, Hogsback Pass (-DB), 15 February 1985, *Phillipson 1030* (PRE). **3327** (Peddie): Near house of No. 6 South Seas, Kap Rivier (-AC), 18 January 2006, *Lubke 5161* (GRA); East London, Blino River Valley, southernwood (-BB), 13 December 1926, *Smith 3627* (PRE); Riet River mouth (-CA), 26 March 1961, *Jacot-Guillarmod 4172* (PRE); Gonubie Park, East London (-DD), 14 April 1968, *Acocks 23929* (PRE). **3424** (Humansdorp): Thuyspunt, S. of Humansdorp (-BB), 11 October 2007, *Low, Walton & Logie ABL15338* (GRA).

ESWATINI: 2531 (Komatipoort): Pigg's Peak District, Komati bridge (-AA), 06 May 1960*, Compton 30052* (NBG; PRE). **2631** (Mbabane): 3 miles [4.82 km] NE. of Forbes Reef (-AA), 13 February 1962, *Schlieben 9548* (PRE); Mbabane, Komati Pass (-AA), *R.H. Compton 31510* (PRE); Mbabane, Ukutula (-AC), 16 February 1956, *Compton 25590* (PRE); Manzini District, Tulwane (-BC), 31 March 1966, *Compton 32546* (PRE); Hlatikulu, Grand Valley Hills (-CD), 23 June 1959, *Compton 28950* (NBG; PRE).

3.3.4. ***Rhynchosia harveyi*** Baker f., Bothalia 1: 121 (1923).

=*Rhynchosia secunda* Harvey, Fl. Cap. 2:251(1862).

Perennial prostrate or climbing herb, up to 0.3 m long. *Stems* trailing on the ground, pubescent to velvety; branches flexuous. *Leaflets* 6–29×6–26 (30) mm, orbicular-rhomboid, obtuse, densely pubescent to velvety; petiole 5–15 mm long; stipules 2–7 × 1–4 mm, broadly ovate, hairy. *Inflorescences* in axillary or terminal racemes, 5–10 cm long, densely pubescent to velvety. *Flowers* 7–10 mm long, yellow with brown tips and reddish veins, pubescent. *Fruits* 12–25×5–8 mm, oblong-falcate, pubescent. Flowering time: from early spring through to late summer (September–February).

Diagnostic characters: *Rhynchosia harveyi*, is characteristically a prostrate or climbing herb with stems trailing on the ground, pubescent to velvety. It can be confused with *R. pentheri* Schltr. and *R. minima* var. *prostrata* due to its silvery to velvet pubescent leaves. However, the leaflets are smaller (6–29×6–26 (30) mm) than *R. minima* var. *prostrata* (10–34×0–35 mm) and the petioles are shorter (5–15 mm versus up to 60 mm). Its distribution is restricted to the Eastern Cape Province while *R. minima* var. *prostrata* is more of an inland species. *Rhynchosia harveyi* has broadly-ovate stipules versus the lanceolate stipules in *R. pentheri*.

Distribution and habitat: *Rhynchosia harveyi* is restricted to South Africa and only found in the Eastern Cape Province (Figure 3.4). It occurs on grassland, savanna and rocky grassland slopes.

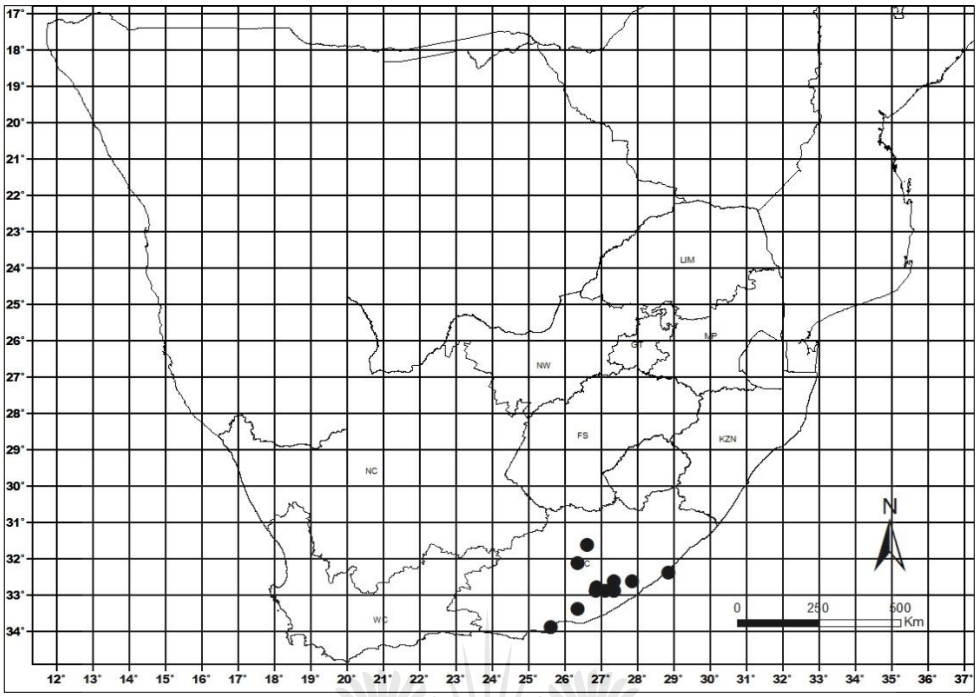

Figure 3.4: Known geographic distribution of *Rhynchosia harveyi* in southern Africa

Conservation status: LC (Raimondo et al., 2009)

Additional specimens examined

SOUTH AFRICA, EASTERN CAPE PROVINCE: 3126 (Queenstown): Plains of Sterkstroom (-DA), 12 February 1958, *Muir 7783* (PRE). **3127** (Lady Frere): Transkei, Xalanga District (-BC), 20 June 1994, *Dold 730* (GRA). **3226** (Fort Beaufort): 6 miles [9.6 km] South East of Tarkastad (-AB), 23 February 1955*, Acocks 17959* (PRE); Alice, Fort Hare, Honeydale Farm, WSW Trollopes Burn-Browse Plots, Control Plot Hillside (-DD), 09 December 1976, *Gibbs Russell 3225* (PRE); Victoria East District, Alice, University of Fort Hare (-DD), 15 April 1935, *Giffen 207* (PRE). **3227** (Stutterheim): Stutterheim (-CB), 11 February 1955, *Adamson D276* (PRE); King William's Town: Perie (-CC), (Precise

date not provided),1901, *Sim 4052* (PRE); King William's Town District, 1 mile [1.6 km] from King William's Town on the East London road (-CD), 21 November 1956, *Comins 1588* (PRE); King William's Town District (-CD), (Precise date not provided) 1901, *Sim 4050* (PRE); Komgha (-DB), December 1891, *Flanagan 1164* (PRE). **3228** (Butterworth): Transkei, Dwessa Forest (-BD), 12 September 1977, *Linder 1201* (PRE); Eliotdale District, The Haven (-BB), 08 October 1966, *Gordon-Gray 787* (NU). **3325** (Port Elizabeth): Alexandria, Port Elizabeth national road (-DC), 27 November 1955, *Archibald 6074* (PRE). **3326** (Makhanda): 2 miles [3.2 km] from Makhanda on Cradock roadside (-AD), 14 December 1977, *Bayliss 8297* (GRA; PRE); Makhanda, (Precise date not provided), *MacOwan 160* (NH); Ecca Reserve (-BA), 03 June 1992, *Chan 187* (GRA); Boknestrand (-DA), 14 April 1995, *Burrows 4351* (GRA).

3.3.5. ***Rhynchosia hirta*** (Andrews) Meikle & Verdc., inTaxon 16:462 (1967); Verdc. in Fl. Trop. E. Afr.: 720 (1971); Drummond in Kirkia 8:226 (1972); Goncalves in Garcia de Orta, Sér. Bot. 5: 106 (1982); Lock, Leg. Afr. Checklist: 430 (1989). *Dolichos hirtus* Andr., Bot. Repos. 7:t. 446 (1807). Type grown in England from seed said to have been sent from the Cape of Good Hope, (Illustration in Andr., Bot. Repos. 7:t. 446 (1807), lectotype, designated by Moteetee et al., 2012).

=*Cyclista tomentosa* Roxb., Pl. Coromandel: 3, t. 221 (1811). *Cyanospermum tomentosum* (Roxb.) Wight & Arn., Prodr. Fl. Ind. Orient.: 260 (1834). *Rhynchosia tomentosa* (Roxb.) Baill., in Bull. Mens. Soc. Linn. Paris I: 387 (1883), non Hook. & Arn. Nec Kurz. Type grown in Calcutta from seed sent from Musore.

=*Cyclista albiflora* Sims in Bot. Mag.:t.1859 (1816). Type grown in England from seeds received from Mauritius.

=*Rhynchosia cyanosperma* Baker in F.T.A.2:218 (1871); Baker f. in J. Linn. Soc., Bot.40:58 (1911); Eyles in Trans. Roy. Soc. South Africa 5:382 (1916); Baker f., Legum.Trop. Africa: 469 (1929). Type: Malawi, Manganja Hills, Meller s.n. (K!, lectotype, designated by Gillett et al. (1971)).

=*Rhynchosia albiflora* (Sims) Alston in Trimen, Handb. Fl. Ceylon 6:85 (1931); Robyns Fl. Sperm. Parc Nat. Alb. 1:348 (1948); Brenan, Checklist For. Trees Shrubs Tang. Terr.:439 (1949); Hauman in F.C.B.6:190 (1954); Meikle in F.W.T.A., ed.2, 1:554 (1958); White, F.F.N.R.: 164 (1962).

Perennial evergreen climbing or twining herb, 2–8 m long. *Stems* are covered with short dark brown hairs when young, and later glabrescent. *Leaflets* broadly elliptic to ovate or almost cordate, terminal leaflets 33–100 (150)×27–65 (110) mm, sparsely pubescent to glabrescent above, densely pubescent with reddish-brown hairs beneath; petiole 15–90 mm long; stipules linear-lanceolate, 6–10×2–5 mm. *Inflorescences* in terminal or dense racemes, 9–22 cm long, reddish-brown pubescent to velvety. *Flowers* 10–25 mm long, varying from blue-mauve or yellow striped with red or pale lemon striped maroon to orange and wine red, glabrous. *Fruits* twisted, narrowly oblong, 20–35×5–9 mm, densely pubescent to velvety. Seeds depressed-globular, brown or shiny dark blue-black. Flowering time: from mid-winter through to early autumn (July–March).

Diagnostic characters: *Rhynchosia hirta*, evergreen climber with leaflets broadly elliptic to ovate, which are characteristically large 33–100 (150)×27–65 (110) mm to all species of *Rhynchosia*. Large flowers (10–25 mm long) with variable colours, blue-mauve or

yellow striped with red or pale lemon striped maroon to orange and wine red. Seeds are distinctly brown or shiny dark blue-black.

Distribution and habitat: *Rhynchosia hirta* is widespread from northeast South Africa northwards into East and Central Africa and westwards into northern Nigeria, and further east to the Mascarene Island, India and Sri Lanka (Fabian and Germishuizen, 1997; Verdcourt, 2001; Moteetee et al., 2012). In South Africa, it occurs the Limpopo and Mpumalanga Provinces (Figure 3.5). It grows in sandy soil in grassland, rocky outcrops, loamy soil or shale in open woodlands and moist forest margins.

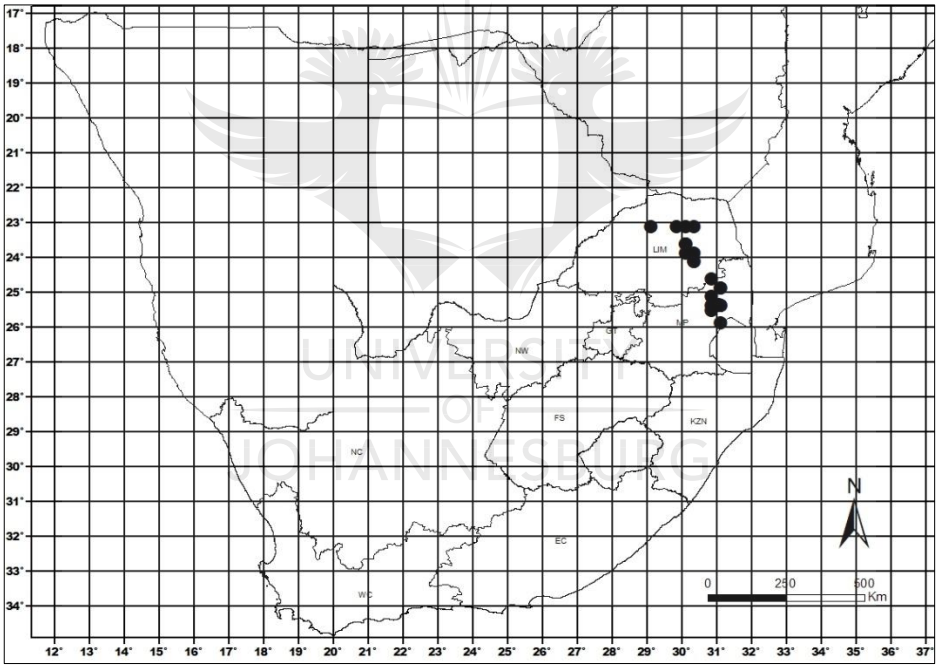

Figure 3.5: Known geographic distribution of *Rhynchosia hirta* in southern Africa

Conservation status: LC (Raimondo et al., 2009)

Additional specimens examined

SOUTH AFRICA, LIMPOPO: 2329 (Polokwane): Louis Trichardt (-BB), December 1933, *Koker 28* (PRE). **2330** (Tzaneen): Soutpansberg District, Elim Hospital, Vutshila Crafts (-AA), *Liengme 145* (PRE); Tshakoma (-AB), November 1931, *Obermeyer TRV30341* (PRE); Westfalia Landgoed, Tzaneen (-CA), 29 March 1967, *Grobbelaar 410* (PRE); Fanie Botha Dam Nature Reserve, *lans ou kanaal* [along the old canal], *bloekombos* [*Eucalyptus*] in Merensky *Grensdraad Wes van Karavaan Park* [Border Wire West of Caravan Park] (-CA), 12 January 1983*, Borchards 51* (PRE); Letaba District, East bank of Ramadiepa River, by rapid falls below Meresky Dam and railway bridge (-CA), 12 October 1959, *Scheepers 722* (BOL); Letaba (-CD), (Precise date not provided), *Renny s.n. PRE 56138* (PRE). **2430** (Pilgrim's Rest): Lekgalameetse Nature Reserve, behind Balloon house no. 10 (-AB), 29 August 1986, *Stalmans 1372* (PRE); Lekgalameetse Nature Reserve, Paris, Road side, along internal fence road (-AB), 23 July 1990, *Stalmans 1997* (NU); Sikosana near Macoutsie River (-AB), December 1922 *Van Dam TRV 30581* (PRE). **MPUMALANGA PROVINCE**: **2430** (Pilgrim's Rest): Blyderivierspoort Nature Reserve, Belvedere Camp, alongside road (-DB), 07 March 2000, *Hankey, Turner & Aubrey 1033* (PRE). **2431** (Acornhoek): 2 miles [3.2 km] North of Bushbuck Ridge (-CC), 18 August 1946, *Codd 1662* (PRE). **2530** (Lydenburg): Sabie, Kiepersol, Eldora, roadside just East of Serra Azul turn-off, (-BB), 27 October 1980, *Deall 42* (PRE); Mataffinkop District (-BD), 22 October 1983, *Onderstall 1064* (PRE); Kaapsehoop road next to Beyth-Lechem (House of Bread) (-DB), 31 January 2019, *Mothogoane 891* (PRE). **2531** (Komatipoort): White River Municipal Town lands (-AC), 10 August 1987*,*

Onderstall 1355 (PRE); White River District, Beersrust 53 Ju. (-AC), 02 September 1983, *Jacobsen 5361* (PRE); Barberton (-CC), November 1932, *Holt 292* (PRE).

3.3.6. ***Rhynchosia resinosa*** (Hochst. ex A. Rich.) Baker, in F.T.A. 2: 218 (1871); Eyles in Trans. Roy. Soc. South Africa 5:382 (1916).; Baker f., Legum. Trop. Africa: 480 (1929); Brenan, Checklist For. Trees Shrubs Tang. Terr.: 440 (1949); Hauman in F.C.B. 6: 162 (1954); Meikle in F.W.T.A., ed.2, 1:554 (1958); White, F.F.N.R.:164 (1962).; Torre in C.F.A.3:318 (1966); Verdcourt in F.T.E.A., Leguminosae, Pap.: 727 (1971); Drummond in Kirkia 8:226 (1972); Jacobsen in Kirkia 9:161 (1973); Goncalves in Garcia de Orta, Sér. Bot. 5:108 (1982); Lock, Leg. Afr. Checklist: 435 (1989). *Fagelia resinosa* Hochst. ex A. Rich., Tent. Fl. Abyss.1:226 (1847).

=*Rhynchosia mildbraedii* Harms, in Mildbraed, Wiss. Ergebn. Deutsch.Zentr. Afrika Exped., Bot.: 267 (1911); Baker f., Legum. Trop. Africa: 480 (1929).

Perennial woody ± erect shrub, climber, or scrambler, often aromatic, 0.4–3 m long. *Stems* are often sticky, covered with dense glandular short hairs, later glabrescent, reddish and fissured. *Leaflets* pale grey in the lower surface, terminal leaflets 35–65×22–65 (70) mm, broadly rhombic or triangular, ovate, distinctly acuminate, rounded at the base, pubescent and sparsely- to very densely-gland-dotted on both surfaces; petiole 20–70 mm long; stipules 3–8×2–4 mm, lanceolate. *Inflorescences* in axillary and terminal racemes, 70–170 mm long, pubescent, glandular and viscid. *Flowers* 12–16 mm long, yellow with purple or reddish streaks, pubescent and glandular. Fruits 21–35 × 7–12 mm, oblong, covered in short and long whitish hairs and glands. Flowering time: from early autumn through to mid-winter (March–June).

Diagnostic characters: *Rhynchosia resinosa* is a robust climber, often aromatic, with sticky stems. The leaves are broadly rhombic or distinctly triangular and acuminate, rounded at the base, paler beneath. The fruit is straw-coloured, with a distinct pale brown reticulation, enclosed in calyx, 21–35×7–12 mm, oblong, covered in short and long hairs. The seeds are distinctly dark reddish-brown marked with black spots.

Distribution and habitat: *Rhynchosia resinosa* is widespread from northern South Africa into Namibia, Botswana (Figure 3.6), Mozambique and Zimbabwe to tropical Africa, including Angola, Sudan, Ethiopia and West Africa (Verdcourt, 2001). In South Africa, it occurs in Limpopo Province only. It grows in river forest margins, limestone areas and in rocky areas.

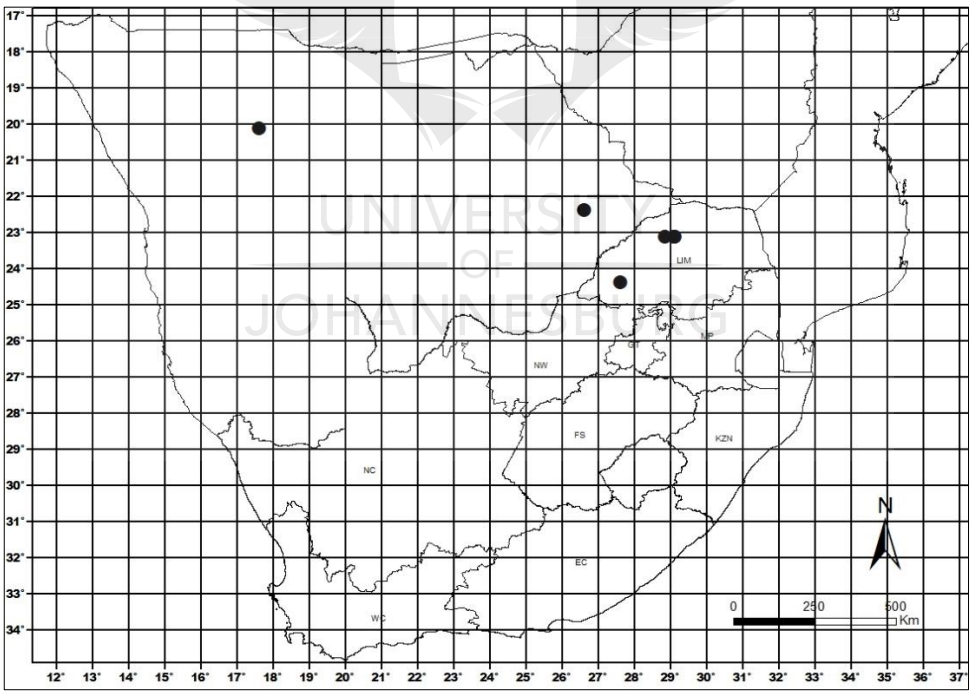

Figure 3.6: Known geographic distribution of *Rhynchosia resinosa* in southern Africa

Conservation status: LC (Raimondo et al., 2009)

Additional specimens examined

SOUTH AFRICA, LIMPOPO PROVINCE: 2329 (Polokwane): Blaauwberg (-AA), 26 April 1961, *Van der Schyff 5413* (PRE); Blouberg, Kloof (-AA), 28 April 1961, *Strey & Schlieben 8599* (PRE). **2427** (Modimolle): Waterberg District, Geelhoutbos (-BC), 11 May 1977, *Germishuizen 342* (PRE).

BOTSWANA: 2226 (Serowe): Kalahari Desert, about 18 miles [28.9 km] West of Serowe (-BC), 08 June 1937, *Erens 201* (PRE).

NAMIBIA: 1918 (Grootfontein): Farm Rankroos 945, on dune south-west of homestead (-BD), 09 April 1990, *Strohbach 998* (WIND). **2017** (Waterberg): *Klipkompleks aan die suidekant van die platorand Waterberg-platopark* [Cliff complex to the south of the escarpment Waterberg plateau], 28 May 1975, *Jankowitz JA600* (WIND); Otjiwarongo

(-AC), 09 March 1971, *Rutherford 89* (WIND); *Suidoostelike kant van Waterberg-platopark* [South-eastern side of Waterberg Plateau Park], Otjiwarongo (-AC), 29 April 1976, *Jankowitz JA851* (WIND); Otjozondjupa, about 12 km north of Okakarara, beyond pump station (-AD), 22 April 2004, *Strohbach BS6015* (WIND). **2019** (Kanovlei): Omaheke District, road to Otjinene (-CD), 14 May 2001, *Uiras MU407* (WIND). **2020** (Eiseb): Omaheke District, against fence to Eiseb Block, ± 1 km north of Otjinoko Omuramba –CA), 18 April 2004, *Strohbach BS6007* (WIND).

3.3.7. ***Rhynchosia sublobata*** (Schum.) Meikle in Kew Bull. 6: 176. Figs.3, 4 (1951); Hauman in F.C.B. 6:155, pl.12 (1954); Meikle in F.W.T.A., ed.2, 1:555 (1958); Torre in C.F.A. 3:313 (1966); Verdcourt in F.T.E.A., Leguminosae, Pap.: 751, figs 109/34, 111 (1971); Drummond in Kirkia 8:226 (1972); Goncalves in Garcia de Orta, Sér. Bot.5:109 (1982); Lock, Leg. Afr. Checklist: 436 (1989). *Glycine sublobata* Schumach. & Thonn., Beskr. Guin. Pl.: 347 (1827); in Kongel. Danske Vidensk. Selsk. Natuurvidensk. Math. Afh.4:121 (1829).

=*Dolichos caribaeus* sensu Hiern, Cat. Afr. Pl. Welw.1:267 (1896), non (Jacq.) Hiern.

=*Rhynchosia caribaea* sensu Baker in F.T.A. 2:220 (1871) prop parte; sensu Harms in Warburg, Kunene-Samb.-Exped. Baum: 264 (1903); sensu Baker f., Legum. Trop. Africa: 474 (1929) pro parte, non (Jacq.) DC.

=*Rhynchosia melanosperma* Klotzch in Peters, Naturw. Reise Mossambique 6, part 1:30 (1861). Type: Mozambique, Sena, *Peters* (B, holotype; K).

=*Rhynchosia memnonia* sensu Hiern, Cat. Afr. Pl. Welw.1:267 (1896) pro parte.

Perennial prostrate or climbing herb, growing from woody rootstock, 0.5– 2 m long. *Stems* glabrescent to densely pubescent. *Leaflets* 17–55×17–60 mm, rhombic, elliptic to ovate, rounded at both ends, often slightly lobed at the base, glabrescent to pubescent, seldom velvety; petiole 15–70 mm long; stipules 2–7×1–2 mm, lanceolate, hairy. *Inflorescences* in axillary racemes and viscous, 6.5–27 cm long, glabrescent or pubescent. *Flowers* 10–15 mm long, yellow or cream-coloured with dark reddish or brown or purple or maroonish stripes, about 12–13 per inflorescence, glabrescent or pubescent. *Fruits* 25–35 × 6–11 mm, oblong-falcate, inflated, slightly pubescent and glandular. Flowering time: throughout the year (January–December).

Diagnostic Characters: *Rhynchosia sublobata*, is a woody prostrate or climber, with stems and rhombic-ovate leaves (covered in hairs). It closely resembles *R. caribaea*, with flowers, yellow or cream-coloured with reddish, brown, purple, or maroonish stripes. However, it can be differentiated by the distinctly inflated fruit, minutely pubescent, while in *R. caribaea*, the fruit is sub-inflated, sparsely pubescent with long silky glandular (golden) hairs.

Distribution and habitat: *Rhynchosia sublobata* is widespread from Namibia, Botswana, northeast South Africa into eSwatini, Mozambique and Zimbabwe, northwards to broader region of Africa, including Central African Republic, Democratic Republic of Congo, Sudan, Somalia, East Africa, West Africa and Yemen (Verdcourt, 2001). It is also found in Madagascar and Comores Islands. In South Africa, it occurs in three provinces (Limpopo, North West, and Mpumalanga) (Figure 3.7). It grows in seasonally swampy grasslands, along rivers or water edges, woodlands, savanna, mountain slopes facing east, among rocks and prefers sandy and turf soils, often on disturbed areas; at altitudes from 10–1300 m.a.s.l.

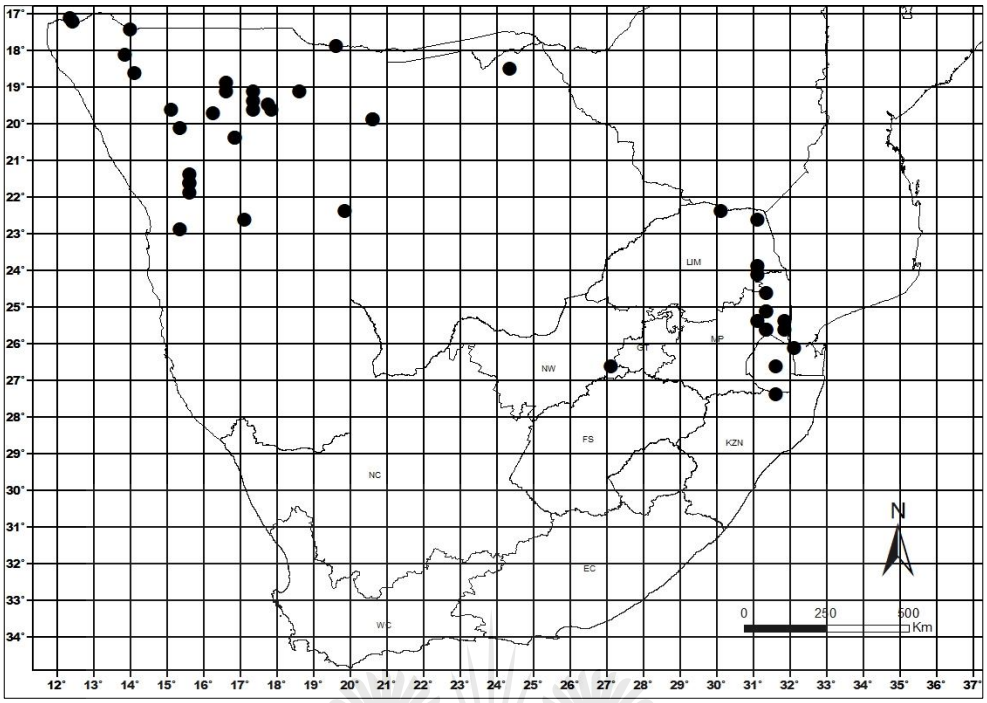

Figure 3.7: Known geographic distribution of *Rhynchosia sublobata* in southern Africa

Conservation status: LC (Raimondo et al., 2009)

Additional specimens examined

NAMIBIA: **1712** (Posto Velho): Kaokoveld, Kunene river at the end of the Marienfluss (-**AB**), 16 May 1991, *Smook 7655* (PRE); Otjinungwa District, 6 km downstream along Kunene River from Otjinungwa (-AB), 06 May 1994, *Kolberg 678* (PRE; WIND). **1714** (Ruacana Falls): Kunene River, 27 km from Ruacana along track to Swartbooisdrif (-AC), 30 April 1994, *Kolberg 665* (PRE; WIND); Omusati District, Mahenene Research Station, edge of cultivated fields (- BD), 08 March 1999*, Kolberg & Loots HK969* (WIND). **1719** (Rantu): Kapako Camp about 4 miles [6.43 km] W. of Mupini Mission (-DC), 07 February 1956, *De Winter* (WIND). **1722** (Singalamwe): Caprivi Strip, Middle West Caprivi (-DC),

19 October 1988, *Maggs GM590* (WIND).**1813** (Ohopoho): Kaokoveld, about 6 miles [9.65 km] W. of Ohopoho (-BB), 28 March 1957, *De Winter & Leistner 5221* (PRE; WIND).**1814** (Tsumeb): Otjikoto (-CA), 11 April 1964, *Giess 9265* (PRE). **1817** (Tsintsabis): Oshikoto, right turn-off from the main road, Road D3001 (-CB), 03 April 2002, *Uiras MU563* (WIND). **1818** (Tsitsib): Oshikoto, Farm Tsutsab area (-CC), 26 February 2001, *Friederich FR12/81* (WIND). **1819** (Karakuwisa): Rudu District, ca. 10 km west of Mile 46 Livestock Development Centre (-AC), 08 February 2006, *Strohbach, Kruger & Kangombe BS6330* (WIND); Nama Pan near Gautscha Pan, E. of Karakuwise (-DC), 23 December 1952, *Maquire 2140* (NBG; BOL). **1914** (Kamanjab): Kunene, Etosha National Park: Kaross, S. of house (-BC), 24 May 2006, *Berriman & Kötting BER2/137* (WIND). **1915** (Okaukuejo): Outjo District, Farm Winkelhaak (-CA), 16 March 1974, *Merxmuller & Giess 30362* (PRE; WIND). **1916** (Gobaub): Gobaub (-BA), 09 March 1976, *Giess & Loutit 14184* (PRE); Cunningham Farm, N. of Outjo (-CB), 12 March 1997, *Germishuizen 9757* (PRE); Namutoni, 18 km from Tsumeb on road to Otavikloof (-DC), 08 February 1974, *Grobbelaar 1888* (PRE). **1917** (Tsumeb): Tsumeb (-AB), 25 April 1978, *Giess 15198* (PRE; WIND); Guinas Lake, on edge, 10 miles [16.09 km] from water pump installation (-AD), 23 February 1989, *Bodenstein 641* (PRE); North of Kombat on road to Gaub Farm (-BC), 03 March 1995, *Germishuizen 7403* (PRE); Otavi (-CB), 05 March 1925, *Dinter 5705* (PRE); Farm Awagobib-GR 45 (-DB), 12 March 1974, *Merxmuller & Giess 30248* (PRE; WIND).**1918** (Grootfontein): Taranaki Boys, 80 km from Grootfontein on road to Rundu (-BA), 05 March 1995, *Germishuizen 7530* (PRE); Plot no.318, farm Groothuis, 3 km from kraal towards homestead (-BC), 23 March 1992, *Strohbach 2016* (WIND); Grootfontein (-CA), (Precise date not provided), *Schoenfelder*

297 (PRE).**1920** (Tsumkwe): Gautscha Pan, 160 miles [257.4 km] E. of Grootfontein (-DC), 21 January 1958, *Story 6233* (PRE; WIND); Nama Pan near Gautscha Pan (-DC), 23 December 1952, *Maguire 2140* (PRE). **2015:** (Otjihorongo): Pamela (-AB), 06 March 1940, *Vlok 2886* (PRE). **2016** (Otjiwarongo): Farm Padberg (-BD), 02 April 1985, *Craven 2119* (PRE); 21.7 miles [34.9 km] E. of Otjiwarango on road to Waterberg (-BD), 16 March 1955, *De Winter 2759* (PRE). **2115** (Karibib): Farm Etemba-Om 135 (-BC), 06 April 1969, *Giess 10800* (PRE; WIND); Omaruru District, Erongo mountains, Farm Schlucht-Om 162 (-DA), 21 March 1965, *Giess 8439* (PRE; WIND); Omaruru (-DA), 21 March 1965, *Tolken & Hardy 765* (PRE); 35 km westich Omaruru (- DA), 21 March 1980, *von Koenen 587* (WIND); Farm Ameib (-DC), 19 March 1963, *Giess, Volk & Bleissner 5922* (PRE). **2116** (Okahandja): Omburo N/O.Omaruru, Basaltfelsen am Omarururivier (-AA), 24 February 1980, *Von Koenen 571* (WIND); 20.4 miles [32.8 km] S. of Okahandja on Windhoek road (-DD), 08 March 1955, *De Winter 2696* (PRE; WIND). **2215** (Trekkopje): Namib Desert Park, Bloedkoppie just west of Tinkas in Namib Desert Park (-CD), 12 May 1976, *Oliver, Muller & Steenkamp 6590* (PRE). **2217** (Windhoek): Munisipale Gebied Windhoek (-CA), 22 November 1962, *Hanekom 131a* (PRE; WIND). **2219** (Sandfontein): Gobabis District, Farm Dawis (-BD), 13 January 1958, *Merxmuller 1205* (PRE; WIND).

BOTSWANA: **1824** (Kachikau): Gubatsa Hills, Linyati, just below summit (-CA), 23 October 1972, *Biegel, Pope & Gibbs Russell 4035* (PRE).

SOUTH AFRICA, LIMPOPO PROVINCE: **2230** (Musina): Soutpansberg District, Musina (-**AC**), December 1917, *Rogers 21211* (PRE). **2231** (Phalaborwa): Kruger National Park**,** Klapperfontein, Langs Spruit (-CA), 18 February 1954, *Van Der Schijff 3556* (PRE). **2331** (Phalaborwa): Skiettocht Military Base (-CC), 11 March 1991, *Manning 718* (PRE). **2431**

(Acornhoek): Phalaborwa District, Phalaborwa Water Board, 14 km S. of Phalaborwa, Farm Sheila 10 KU (-AA), 30 October 1986, *Retief 487* (PRE). **NORTH WEST PROVINCE**: **2627** (Potchefstroom), ±4 km from Potchefstroom on Vredefort road, near fence on slope (-CA), 08 March 1996, *Cilliers 2500* (PRE). **MPUMALANGA PROVINCE**: **2431** (Acornhoek): Pilgrim's Rest District, Hermitage, Manyeleti Game Reserve (-CB), 22 March 1977, *Bredenkamp 1796* (PRE). **2531** (Komatipoort): Kruger National Park, Pretoriaskop (-AB), 04 February 1953, *Van der Schijff 2028* (PRE); 16 km from Kaapmuiden on road to Mbombela via Kanyamazane (-AC), 12 March 1989, *Germishuizen 5157* (PRE); 16 km from Kaapmuiden, N4 road to Mbombela via Kanyamazane (-AC), 31 January 2019, *Mothogoane 890* (JRHAU; PRE); 46 km from Barberton turnoff on road to Kaapmuiden (-CB), 15 August 1985, *Germishuizen 3303* (PRE); Kaapmuiden, 2 km from Kudu Lodge on road to Komatipoort (-CB), 11 March 1989, *Germishuizen 5126A* (PRE). **2731** (Louwsburg): Piet Retief District, Near Pongala bridge and Pongola Village-Magate Road (-BC), 09 March 1964, *Edwards 3266* (NU; PRE). **KWAZULU-NATAL PROVINCE**: **2632** (Bela Vista): Ingwavuma District, Ndumo Game Reserve, Pongola River (-CD), 09 December 1971, *Pooley 1555* (NU).

ESWATINI: **2631** (Mbabane): Hlatikulu District, Near Sipofaneni (-DA), 03 March 1960, *Compton 29892* (PRE). **2632** (Mbabane): Blue Jay Ranch, in valley of Umbuluzi River in Lubombo Mountains, 2 miles [3.21 km] upstream, W. of Mozambique border (-AA), 31 July 1977, *Culverwell 938* (PRE).

CHAPTER 4: ETHNOBOTANY, ANTIMICROBIAL ACTIVITY AND PHYTOCHEMISTRY OF MEDICINAL SPECIES OF *RHYNCHOSIA* IN SOUTHERN AFRICA

4.1 Introduction

4.1.1 Ethnobotany of *Rhynchosia*

Despite the fact that *Rhynchosia* is a relatively large and widely-distributed genus, no ethnobotanical studies have been undertaken on the genus as a whole, be it regionally or globally. However, a number of species have been reported to have ethnobotanical uses across the globe, but the information is scattered in the literature. For example, in India, the leaves of *Rhynchosia beddomei* Baker and *R. heynei* Wt. & Arn. are reportedly used in the treatment of skin conditions, rheumatism, and arthritis (Ram et al., 2004; Bhakshu and Raju, 2009); *R. bracteata* Benth.ex Baker, *R. capitata* (Roth) DC*., R. minima* and *R. scarabaeoides* (L.) DC are used as remedies for gastro-intestinal ailments such as dysentery and diarrhoea (Patil and Bhaskar, 2006; Challa et al., 2011; Singh et al., 2013), whereas the seed of *R. rufescens* (Wild) DC. is used as an energy booster (Gritto et al., 2015). The seeds of *Rhynchosia nulubilis* (Black Soybean), are used to enhance body fluid circulation, detoxificafion and as an anti-inflammatory in China (Yim et al., 2009). In Mexico, the seeds of *R. pyramidalis* (Lam.) Urb., are used as a topical analgesic, while in the Dominican Republic, it is used as a male aphrodisiac and also as a tea or alcohol beverage (Cleversley, 2002). The seeds of *Rhynchosia volubilis* Lour. are used to alleviate coughs in Japan (Kinjo et al., 2001). The leaves of *R. viscosa* DC. are used as an abortifacient and inducement of labour by the rural communities in western Uganda, whereas in Kenya, it is used to treat itchy rashes (Kokwaro, 1976;

Stangeland et al., 2011). The root of *R. insignis* (O. Hoffm.) R.E.Fr. in Zimbabwe, is used for the alleviation of abdominal pains, treatment of depressed fontanelle in infants and dropsy (swollen stomach) (Gelfand et al., 1985). In addition to the above mentioned uses, *R. minima* is also used as an abortifacient (Yellasubbaiah et al., 2015; Basha and Reddy, 2017), antihelminthic, wound healing, asthma and in the treatment of piles (Yellasubbaiah et al., 2015). In this study, we investigated the ethnobotany of medicinally important *Rhynchosia* species that occur in southern Africa.

4.1.2 Antimicrobial activity of *Rhynchosia*

Notably, not much antimicrobial work has been done on *Rhynchosia* in southern Africa, but there are a number of *Rhynchosia* species around other parts of the world that have been studied. The crude extract of *Rhynchosia reniformis* DC. recorded a 100% inhibition of *Streptococcus cricitus*, whereas the ethyl acetate fractions exhibited a noteworthy activity against *Micrococcus flavas* (99% inhibition), *Streptococcus cricitus* (95% inhibition) and *Pseudomonas morganii* (90% inhibition) (Shinwari et al., 2013). *Rhynchosia scarabaeoides* (L.) DC., used traditionally in India for dysentery, showed noteworthy activity against *Staphylococcus aureus* and *Proteus vulgaris* (Challa et al., 2011). According to Bhakshu and Raju (2009), the essential oil of *R. heynei* Wright & Arn. was observed as very effective against *Pseudomonas aeruginosa* and *Micrococcus luteus*. *Rhynchosia capitata* has demonstrated good inhibition properties against *S. aureus* and *Klebsiella pneumoniae* (Praveena et al., 2014). The study by Gundidza et al. (2009), which investigated essential oils of *R. minima* demonstrated antibacterial activity against *C. albicans* and *Aspergillus flavus*. The high antifungal activity may be attributed to the presence of O-cymene and limonene in the essential oil, which have been shown

to have strong antifungal properties (Gundidza et al., 2009). The essential oil from *R. minima*, also showed anti-oxidant activity (Gundidza et al., 2009). An alcoholic extract of *R. minima* exhibited antimicrobial activity against *Staphylococcus aureus*, *Neisseria gonorrhoeae* and *Shigella boydii* (Chhabra and Mahunnah, 1994). The antimicrobial activity of the southern African selected species tested is presented here.

4.1.3 Phytochemical properties of *Rhynchosia*

The biological activities of a plant used as medicine, are due to its phytochemical composition (Ahmad et al., 2016). *Rhynchosia capitata* (Roth) DC., which is found to have good antioxidant activity, has been reported to have flavonoids, tannins and saponins (Chaturvedi, 2015). Phytochemical studies have also shown that *Rhynchosia* species such as *R. minima* contained essential oils (Gundidza et al., 2009), tannins, flavonoids and triterpene steroids (Jia et al., 2015).Tannins, flavonoids and triterpene steroids may act against colon cancer (Jia et al., 2015). In addition, flavonoids may also reduce inflammation, as well as carcinogenesis (Morris, 2008). These compounds have demonstrated a wide range of pharmacological activities against most bacterial species such as *Clostridium perfringens*, *Klebsiella pneumoniae*, and others. Phytochemical analysis in duirectic activity of *R. beddomei* by Eswaraiah et al. (2013), listed alkaloids, amino acids, carbohydrates, carotenoids, sterioids, tannins, and sesquiterpenes as chemical compounds present in the plant, which are believed to be responsible to induce diuresis. In Korea, *R. nulubilis* seeds are reported to have immune-modulating properties, are very effective against tumars, and anthocyanins present in the seed provides effective protection (Park and Lee, 2013). In a study by Rondo (2017), *Rhynchosia villosa*, a plant that is used in the treatment of skin conditions, improvement of skin texture and tone in

the Eastern Cape, was found to have five types of flavonoids (Genistein, cajacin, 2' hydoxygenistein, catechin and gallocatechin), and these chemical compounds were successfully isolated. The study also found that the root extract of this plant inhibited mushroom tyrosinase activity at 15.67 ug/ml. Jia et al. (2015), indicated that *Rhynchosia* species have already demonstrated antimicrobial and anthelmintic activities, but there is little information on their anti-cancer activity. The qualitative phytochemical screening of seleted *Rhynchosia* is discussed in detail further on.

4.2 Materials and methods

4.2.1 Ethnobotany

A literature survey of *Rhynchosia* species that occur naturally in southern Africa was conducted to collate ethnobotanical knowledge, with regards to their medicinal significance from published and unpublished papers. Over 60 literature sources were consulted, including books, journals, and internet sites.

4.2.2 Collection of plant material

Fresh plant specimens of selected *Rhynchosia* species were collected during autumn and spring seasons from natural populations around Pretoria in Gauteng Province and also Mpumalanga Province (Table 4.1, 4.5). The identifications of these specimens was done by the author at the National Herbarium (PRE) of the South African National Biodiversity Institute (SANBI). The herbarium voucher specimens collected during field trips are housed at JRAU and their duplicates are deposited at PRE. The species used for this study were selected on the basis of documented traditional use and the accessibility to the localities. Unfortunately, due to unavailability of plant material (despite repeated

attempts to locate them in the field), as well as time constraints, only three species were tested for their antimicrobial activity. The plants were washed with tap water, cut into small pieces, air dried for two weeks and then ground into fine powder using an electric blender, for both phytochemical and antimicrobial screening (One population was studied for each species tested) .

4.2.3 Antimicrobial screening

4.2.3.1 Sample extraction

The extraction of samples for antimicrobial screening was performed using (i) organic solution consisting of dichloromethane (DCM) and methanol at a ratio of 1:1, and (ii) distilled water for aqueous extraction.

In preparing organic extracts, an empty flask was first weighed, labelled and the weight recorded, plant material placed in the flask, weighed and the total mass recorded. The solution of dichloromethane and methanol was added to the ground plant material, sealed with foil to minimise evaporation and then put in the shaker incubator at 37˚C for 24 hours. After the 24-hour duration the supernatant was poured into a beaker. The remaining pellet from the first mixture was then re-added with DCM solution at a ratio of 1:1, and incubated for another 24 hours. The second supernatant was then added to the first and put underneath a fume hood to dry for five days. For the effective transfer of dry mass solution into a weighed and labelled vial, acetone was added into the beaker with the residue and then transferred into the vial with the total mass recorded. Once the acetone had evaporated from the vial (after three days), the dry mass was weighed and the yield determined.

In preparing the aqueous extracts, an empty flask was weighed, weight recorded, and then the plant material was placed in the flask, and the total mass recorded. Sterile distilled water was then added at an equal volume to the ground powdered plant material and put in shaker incubator at 30˚C for 24 hours. The supernatant was then transferred into a previously weighed and labelled vial, and then frozen at -80˚C for 48 hours. To drain all the moisture, the vial was then freeze dried in a lyophilizer and the final residue weighed and recorded to determine the yield. Ten plant extracts were prepared using water (sterile) and dimethyl sulfoxide (DMS) from one population of each species, plants parts of *Rhynchosia adenodes* (roots), *R. hirta* (leaves and roots) and *R. sublobata* (leaves and roots).

Table 4.1: The calculation of medicinal plant yields used for antimicrobial screening.

Species name	Voucher number	Parts used	Extraction	Empty flask(A) (g)	Flask + plant material (B) (g)	Empty vial (C) (g)	Vial + dry mass (D) (g)	Plant material (B-A = E) (g)	Extract (D-C=F) (g)	% yield (F/E× 100)
Rhynchosia adenodes	MSM & TTM 06 (JRHAU; PRE)	Roots	Organic	113.02	149.01	32.6289	36.2024	35.99	3.5735	9.92
			Aqueous	105.85	123.29	8.5622	8.9154	17.44	0.3532	3.96
Rhynchosia hirta	MSM891 (JRHAU; PRE)	Leaves	Organic	106.54	124.40	31.8958	33.5110	17.86	1.6152	9.04
			Aqueous	106.55	111.73	8.5622	8.9168	5.18	0.3546	6.84
		Roots	Organic	93.73	122.29	32.7822	39.5877	28.56	6.7455	23.56
			Aqueous	113.02	130.58	8.5622	8.9607	17.56	0.3985	2.26
Rhynchosia sublobata	MSM890 (JRHAU; PRE)	Leaves	Organic	118.86	150.61	32.7039	35.0004	31.75	2.2965	7.23
			Aqueous	94.89	131.60	8.5622	8.9611	36.71	0.3989	1.08
		Roots	Organic	105.86	153.58	32.8674	36.6427	47.72	3.7753	7.91
			Aqueous	93.78	135.54	8.5622	9.5263	41.76	0.9641	2.30

4.2.3.2 Selection of bacteria for antimicrobial screening

The selection of bacteria was based on the ethnobotanical uses (Table 4.2).

Table 4.2: The selected pathogens used for antimicrobial activity assessments.

Category of ailments	**Pathogens and their strain number**
Gastro-intestinal disorders	*Enterococcus faecalis* (ATCC29212) *Escherichia coli* (ATCC8739) *Staphylococcus aureus* (ATCC25923)
Respiratory infections	*Pseudomonas aeruginosa* (ATCC27853)
Urinary tract infection (UTI): Bladder	*Proteus vulgaris* (ATCC33420)

4.2.3.3 Culture preparation

Antimicrobial activity

In making preparations for minimum inhibition concentration (MIC), a starting concentration of 32 mg/ml was determined using the extraction yield and the amount of sterilised water or DSMO that had to be added. To eliminate contamination during the assay, the lamina flow cupboard was sprayed with ethanol (76%) and wiped with an autoclaved cotton wool. The microplates were all numbered appropriately. The culture control was left overnight at room temperature and was used as an indicator for contamination by the presence of murkiness. All 96 wells were filled with 100µg of Tryptone soya broth (TSB), using multi pipette. Thereafter, plant extracts and two controls

(Antibiotic Ciprofloxacin (Sigma) at a starting concentration of 0.01 mg/ml, as a positive control and acetone (prepared with sterilised cold water), as a negative control, at the same concentration as the plant extracts) were added to the micro-titre plates, serial dilutions were performed, to reduce the concentrations of plant to 16 mg/ml. The prepared microplates were all inoculated with 100uq of the selected pathogen. To check the vialibity of the bacteria, the bacteria was streaked on argon media plates and incubated along with the plates. To reduce evaporation, all plates were sealed with sterile adhesive sealer and incubated for 24 hours at 37 ^{0}C. After 24 hours of incubation, 40 ul of the colour indicator, p-iodonitrotetrazolium iodide (INT) was added to each well, then after 4 hours, detection of results was performed. The pink colour formation on any well on the micro-titre plates indicated bacterial growth, and no pink colour indicated antimicrobial activity of the plant extracts. The microplates were examined on a light box and results recorded on the template sheet. The MIC values were determined according to the method described by Eloff (1998). The antimicrobial activity of the extracts against the pathogens was classified according to MIC values (following Kuete, 2010; Pauw and Eloff, 2014) as follows:

≤ 0.16 mg/ml = noteworthy activity

> 0.16 ≤ 0.625 = moderate activity

≥0.625 = low activity

4.2.4 Phytochemical screening

Phytochemical screening was conducted according to extraction procedures and detection methods by Tiwari et al. (2011) and Zohra et al. (2012).

4.2.4.1 Preparation of plant extracts

Plant parts (leaves and roots) of selected species of *Rhynchosia* were cut into small pieces, dried at room temperature and then ground into fine powder (Table 4.5). The plant extracts were prepared using distilled water, acetone, methanol and ethanol (for coumarins); 5 g of powdered plant material was mixed with 50 ml of each solvent. The mixture was left for 24 hours at room temperature and covered with Para-film to prevent evaporation. The extract solution was filtered using Whatman No.1 filter paper, using Büchner funnel. The filtrates prepared from various solutions were weighed, recorded and then stored at 4˚C until further use. The extracts were then screened for the presence of alkaloids, flavonoids, terpenoids, saponins, tannins, coumarins, and phenols using detection methods outlined below.

4.2.4.1.1 Alkaloids (Tiwari et al., 2011)

Alkaloids test was performed using Wagner's Test, wherein 5 ml of the extract was dissolved in diluted hydrochloric acid (HCl) and filtered. The filtrate was then treated with Wagner's reagent which is composed of 1.27 g of iodine and 2 g of potassium iodide in 100 ml of distilled water. The formation of reddish-brown precipitate indicated the presence of alkaloids.

4.2.4.1.2 Flavonoids (Tiwari et al., 2011)

The detection of flavonoids was performed using the Alkaline Reagent Test. The filtrates were treated with a few drops of sodium hydroxide solution. With the addition of few drops of diluted HCl turned the intense yellow solution colourless indicated the presence of flavonoids.

4.2.4.1.3 Terpenoids (Tiwari et al., 2011)

Salkowki's test was performed to detect the presence of terpenoids. One millilitres of chloroform was added to 2 ml of the filtrates, followed by a few drops of concentrated sulphuric acid. The presence of terpenoids was indicated by the immediate production of reddish-brown precipitate.

4.2.4.1.4 Saponins (Tiwari et al., 2011)

The detection of saponins was performed using the Foam Test. 0.5 g of the extract was mixed with 2 ml of water, shaken vigorously, and allowed to stand for ten minutes. The presence of saponins was indicated by the presence of the foam that persists even after ten minutes.

4.2.4.1.5 Tannins (Tiwari et al., 2011)

The Gelatine Test was performed to detect the presence of tannins. One percent of gelatine solution containing sodium chloride was added to the filtrate. The formation of white precipitate indicated the presence of tannins.

4.2.4.1.6 Coumarins (Zohra et al., 2012)

The detection of coumarins was performed by evaporating 5 ml of ethanolic solution and then dissolving the residue (solute) in 1–2 ml of hot distilled water. The solution was divided into two halves, one half served as control, while 0.5 ml of 10% ammonium hydroxide was added to the other half. Two drops of each solution were blotted on filter paper and examined under UV light. Intense fluorescence indicated the presence of coumarins.

4.2.4.1.7 Phenols (Tiwari et al., 2011)

The Ferric Chloride Test was used for the detection of phenols. The filtrate was treated with 3 to 4 drops of ferric chloride solution. The formation of bluish-black colour indicated the presence of phenols.

4.3. Results and Discussions

4.3.1. Ethnobotany

The results of the literature survey on the ethnobotanical uses of *Rhynchosia* species occurring in southern Africa are summarised in Table 4.3. It should be noted, however, that the uses are not only limited to the region. The survey has revealed that there are 10 species of *Rhynchosia* (*R. adenodes* Eckl. & Zeyh., *R. albissima* Gand., *R. caribaea* (Jacq.) DC., *R. densiflora* (Roth) DC., *R. harveyi* Eckl. & Zeyh., *R. hirta* (Andrews) Meikle & Verdc., *R. minima* (L.) DC., *R. resinosa* (Hochst. ex. A. Rich.) Baker, *R. sublobata* (Schumach. & Thonn.) Meikle), and *R. villosa* (Meisn.) Druce) that have been recorded to be used medicinally for treating various infections.

Table 4.3: Ethnobotanical information of medicinally important *Rhynchosia* species

Botanical (Scientific) name	Vernacular names	Plant part(s) used	Medicinal use (country/region)	Mode of administration	References
R. adenodes Eckl. & Zeyh.	*Monya-mali, monyamadi* (S) ; *ungazini* (Z)	Roots	To treat rheumatic pains, menstrual pains and dysentery (Lesotho)	Oral	Shale et al. (1999); Moffett (2010); Moteetee and Van Wyk (2011); Stark et al. (2013); Possa and Khotso (2015)
R. albissima Gand.		Roots	Aphrodisiac, male impotence (fertility) and as a purgative; to induce delivery (mixed with *Tetracera parviflora* (Rusby) Sleumer (=*T. aspera* var. *boliviana* Kuntze) (India, Tanzania)	Oral	Hedberg et al. (1983); Neuwinger (2000); Lima et al. (2014)
R. caribaea (Jacq.) DC.	*Monya-mali*, *monyamadi*, *morarawaiphepha*, *morarana-oa-liphepa*, *thara* (S); *isihlahlasenqomfi* (Z)	Roots	Headaches (dried powdered roots are sniffed), rheumatic (lotion of cooked roots is applied) (Lesotho)	Inhalation, topical	Moffett (2010); Moteetee and Van Wyk (2011)
Rhynchosia densiflora (Roth) DC.		Roots	Vomiting and diarrhoea (Ethiopia); dysentery (Northern Uganda)	Oral	Okello and Ssegawa (2007); Lulekal et al. (2008)
Rhynchosia harveyi Eckl. & Zeyh.	*Iyezalesisusegazi*, *uvuma; umhlunguthi* (X)	Roots	Headache (Eastern Cape)	Nasal	Dold and Cocks (1999); Neuwinger (2000)
R. hirta (Andrews) Meikle &Verdc.		Leaves, roots	Stomach disorders (Kenya, Pakistan); otitis, sciatic pain syndrome, elephantiasis (Africa); for a quick painless delivery, to expel placenta, schistosomiasis (bilharzia), purgative, ulcers, cancer, malaria (Kenya)	Oral, topical	Kokwaro, (1976); Neuwinger (2000); Jeruto et al. (2010; 2011); Amjad et al. (2015)
R. minima (L.) DC.	*Iyezalesisu* (X)	Whole plant, roots, leaves	To cure chronic fever, abortifacient, anthelminthic, treatment of wounds, asthma and piles (India); skin conditions (Zimbabwe, Qatar), boils (Zimbabwe, China), respiratory infections (root) (China); tuberculosis, venereal diseases, urinary bladder, kidney complains and diarrhoea (India); menopausal problems;	Oral, topical	Chhabra and Mahunnah (1994); Dold and Cocks (1999); Neuwinger (2000); Mali et al. (2006); Singh (2006); Morris (2008); Gundidza et al. (2009); Jain et al. (2009); Chaudhary (2010); Rothe (2011); Kanthale and Biradar (2012); Kumar et al. (2012); Maheswari

			antibiotic (USA); gonorrhoea (Tanzania); cardiac pain, schistosomiasis (bilharzia), diarrhoea (Africa)		et al. (2012); Singh et al. (2013); Rekha et al. (2014); Jia et al. (2015); Johnson et al. (2015); Yellesubbaiah et al. (2015); Kshirsagar (2016); Kumudha and Yogeshwari (2016); Phondani et al. (2016); Basha and Reddy, (2017)
R. resinosa (Hochst. ex. A. Rich.) Baker		Roots, branches, whole plant, leaves	Diarrhoea (Uganda, Zimbabwe); abdominal pains, dysmenorrhea, to expel maggots from wounds (whole plant), abdominal pain in pregnant women (root), to dilate the birth canal (roots, leaves) and sterility (branch) (Zimbabwe); syphilis (leaves) (Southern Uganda); peptic ulcers (Together with *Ozoroa insignis* Delile, *Gymnosporia senegalensis* (Lam.) Loes, *Entada abyssinica* Steud. ex A. Rich. and *Lannea schimperi* (Hochst. ex A. Rich.) Engl.) (Tanzania, India) Wound dressing (leaf) (Africa)	Oral	Gelfand et al. (1985); Anokbonggo et al. (1990); Neuwinger (2000); Ssegawa and Kasenene (2007); Barik et al. (2014)
R. sublobata (Schumach.) Meikle		Leaves, stem, bark, roots	Tachycardia, gall bladder disorders in children (Tanzania); diarrhoea, dizziness, urinary incontinence, tooth diseases, pneumonia, swollen knees, scabies, headache and treatment of snake bite (Africa); dysentery (Mozambique); chest illness (Tanzania); stomach problems (Tanzania/Mozambique), antidote for snake (root and bark) (Tanzania)	Oral, topical	Kokwaro (1976); Neuwinger (2000); Mageresi et al. (2008); Bruschi et al. (2011)
R. villosa (Meisn.) Druce	*Uphuzi lobala*	Leaves, roots	Used externally and internally to improve skin texture and tone; soaked leaves are used to wash swollen body organs as treatment.; used in hormone dependent diseases	Oral, topical	Rondo (2017)

Based on the recent checklist, though outdated, there are about 69 *Rhynchosia* species occurring in southern Africa (Germishuizen, 2006), it is surprising that only a few species have been recorded to be used for medicinal purposes. *Rhynchosia minima* is the most widespread species (southern Africa, throughout the tropical Africa, Cape Verde Islands, middle east countries, India, China and also in tropical America), therefore it is not surprising that it is the most cited species, especially in Asian countries such as India and China (Mali et al., 2006; Jain et al., 2009; Chaudhary, 2010; Rothe, 2011; Kanthale and Biradar 2012; Kumar et al., 2012; Rekha et al., 2014; Jia et al., 2015; Yellesubbaiah et al., 2015; Johnson et al., 2015; Kshirsagar et al., 2016; Kumudha and Yogeshwari 2016; Basha and Reddy, 2017), whereas *R. caribaea* (occurs in Namibia, Botswana, South Africa: Limpopo, North West, Gauteng, Mpumalanga, Free State, KwaZulu-Natal, Western Cape and Eastern Cape Provinces, and also in eSwatini), *R. densiflora* (found in Namibia, Botswana, South Africa's provinces: Limpopo, North West, Gauteng, Mpumalanga and KwaZulu-Natal, and also in eSwatini), *R. harveyi* (only found in the Eastern Cape Province) and *R. villosa* (Restricted to South Africa: Mpumalanga, KwaZulu-Natal and Easten Cape Provinces) appear in a few records only as shown in Figure 4.1.

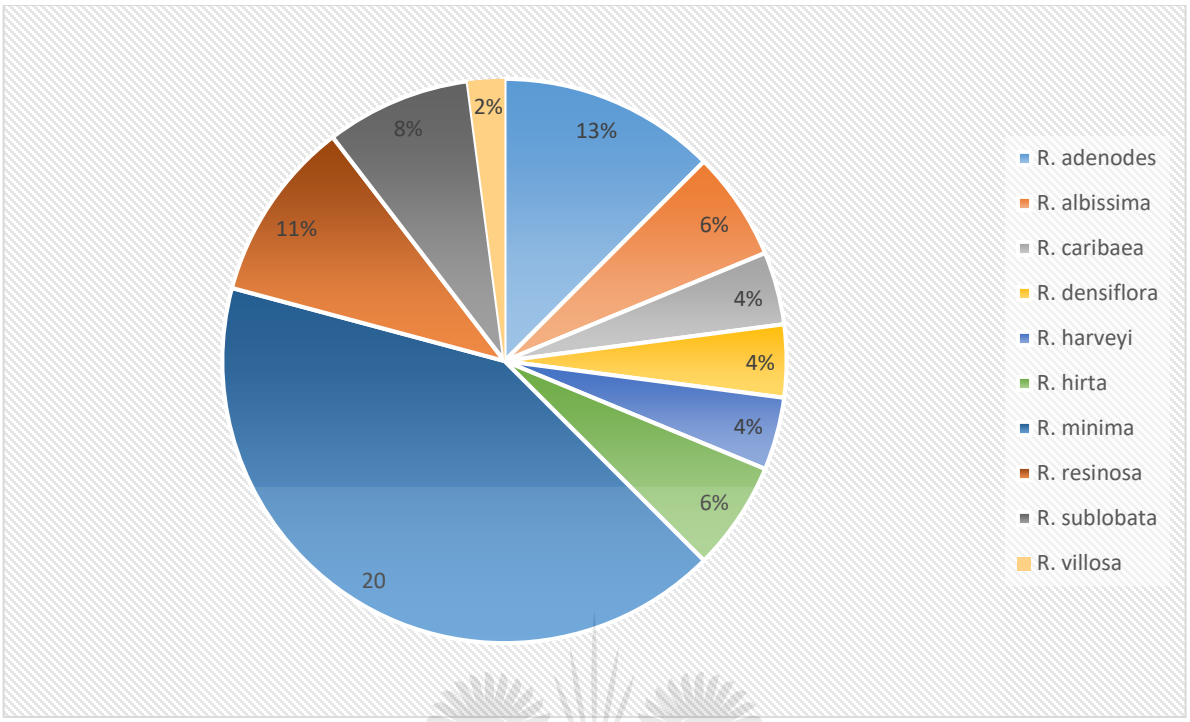

Figure 4.1. Representation of number of references of southern African *Rhynchosia* species uses as published in the literature.

Preference on the usage of plant parts was given to roots (56%), leaves (35%), stems (7%), and branches (2 %) as indicated in Figure 4.2. The high preference of using roots and leaves has also been reported in other studies (Semenya et al., 2012; Mahwasane et al., 2013; Semenya and Maroyi, 2013; Afolayan et al., 2014; Hughes et al., 2015). The widespread use of roots for medical preparations is mainly based on people's strong beliefs that underground parts act as reservoirs of several healing agents and are considered to possess greater healing power than other plant parts (Semenya et al., 2012; Mahwasane et al., 2013; Hughes et al., 2015). The roots are mostly preferred in traditional medicine, partly because of their availability in all seasons of the year (Mahwasane et al., 2013). This is of great concern, as the harvesting of underground parts (roots, bulbs, etc.) is very destructive and unsustainable for the future survival of

the species and the use of alternative parts such as leaves is encouraged (Zschocke et al., 2000; Moeng and Potgieter, 2011).

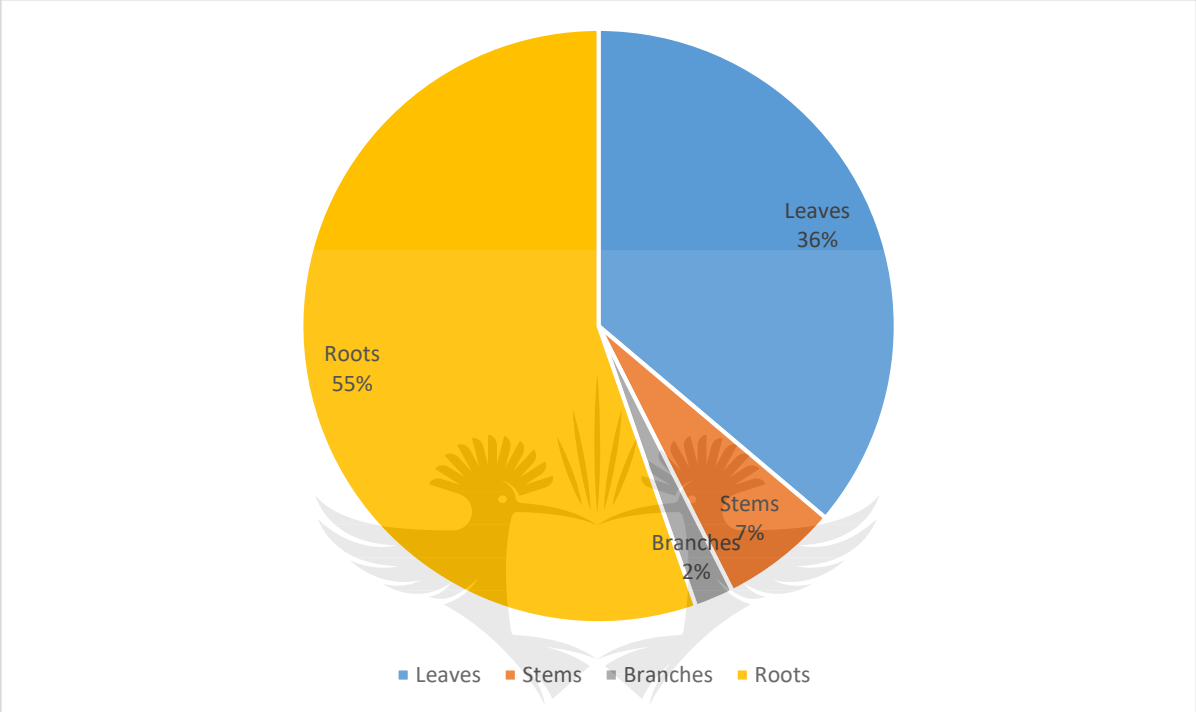

Figure 4.2: A diagram representing plant parts of *Rhynchosia* species used medicinally in the treatment of illnesses.

Interestingly, although according to literature records ten *Rhynchosia* species which occur naturally in southern Africa are medicinally used (Table 4.3), only four (i.e. *R. adenodes*, *R. caribaea, R. harvey*i and *R.villosa*) have been reported to be used in southern Africa (Dold and Cocks, 1999; Shale et al.,1999; Neuwinger, 2000; Moffett, 2010; Moteetee and Van Wyk, 2011; Stark et al., 2013; Possa and Khotso, 2015; Rondo, 2017), while the remaining species are used mainly in Asia (e.g. India, China, etc.) and East Africa. The recorded medicinally utilised *Rhynchosia* species in southern Africa, are mainly used for the treatment of infectious diseases falling into the following four

categories: gastrointestinal, respiratory tract infections (RTIs), skin conditions, and sexually transmitted infections (STIs) (Figure 4.3). Six *Rhynchosia* species (*R. adenodes*, *R. densiflora*, *R. hirta*, *R. minima*, R*. resinosa* and *R*. *sublobata)* are used to treat gastrointestinal disorders such as diarrhoea and stomach-ache (Table 4.3). Two species (*R. minima* and *R*. *sublobata*) are utilised for the treatment of RTIs including coughs, colds and asthma (Table 4.3). Four species (*R. minima,* R*. resinosa, R*. *sublobata* and *R. villosa*) are exploited for the alleviation of skin conditions such as boils, promotion of healing of wounds and scabies (Table 4.3). Two species (*R. minima* and R*. resinosa)* are utilised for the treatment of STIs including syphilis, gonorrhoea, etc. (Table 4.3).

These plants are also used as aphrodisiacs (Neuwinger, 2000) and for the treatment of rheumatic pains, headaches (Watt and Breyer-Brandwijk, 1932; Shale et al., 1999; Neuwinger, 2000), male impotence (Gelfand et al., 1985; Neuwinger, 2000), menstrual pains (Gelfand et al, 1985; Shale et al., 1999; Neuwinger, 2000), elephantiasis (Neuwinger, 2000), bilharzia, and otitis (Kokwaro, 1976; Neuwinger, 2000), and also for a quick painless delivery (Kokwaro, 1976; Gelfand et al., 1985; Neuwinger, 2000), to expel placenta (Neuwinger, 2000), as antidote for snake bites (Kokwaro, 1976).

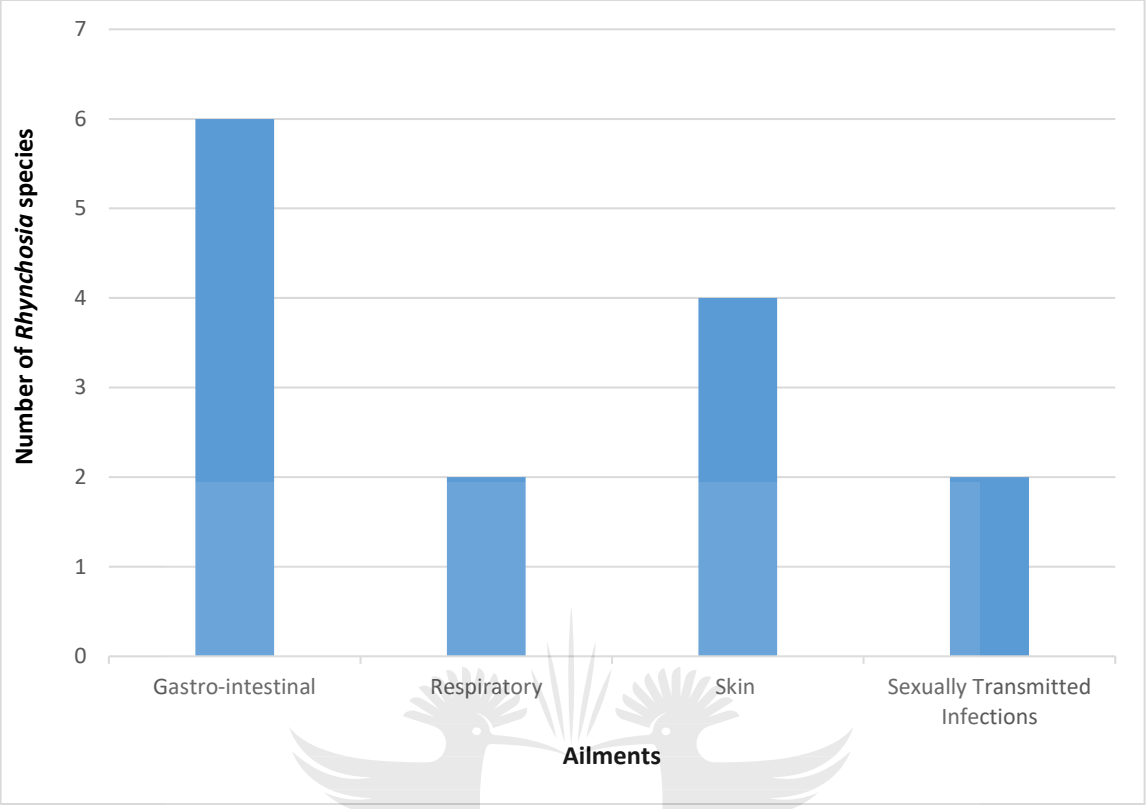

Figure 4.3: Diagram showing the number of southern African *Rhynchosia* species utilized for the treatment of various ailment classes.

Rhynchosia adenodes is one of the most commonly used species by different tribes in southern African countries such as in Lesotho and South Africa. Some of the Nguni speaking tribes residing in South Africa, i.e. the Zulus and Swazis, use the leaf infusion of this species for dysentery, whereas the Basotho use crushed roots and stem mixed with milk as a remedy for dysentery in calves and cattle (Watt and Breyer-Brandwijk, 1932; Loeb and Loeb, 1956; Shale et al., 1999; Moffett, 2010; Moteetee and Van Wyk, 2011; Stark et al., 2013). The Basotho people also use root decoction in the treatment of rheumatism and menstrual pains (Shale et al., 1999; Moteetee and Van Wyk, 2011; Stark et al., 2013; Possa and Khotso, 2015). According to Possa and Khotso (2015), it is believed that the medicine cleanses the womb and is given to barren women to help treat

infertility. This species is also one of the most widely distributed in the region, which might explain its popularity.

In Northern and Eastern Africa, a root decoction of *R. albissima* is used to treat male impotence, as an aphrodisiac and purgative (Neuwinger, 2000). When used as an aphrodisiac, the dried pulverised root is mixed with porridge and given to the patient. In Tanzania, when mixed with *Tetracera parviflora* (Rusby) Sleumer (=*T. aspera* var. *boliviana* Kuntze*)*, root decoction is used to induce labour in a woman who has been pregnant for more than the nine months period and is unable or has difficulty in going into natural labour (Hedberg et al., 1983; Lima et al, 2014).

The Basotho people, use the root decoction of *R. caribaea* as a lotion (from cooked roots) for the treatment of rheumatic pains (Watt and Breyer-Brandwijk, 1932; Moffett, 2010; Moteetee and Van Wyk, 2011). The powdered root is used as snuff to relieve headaches, the decoction of the plant was in the past used to wash garments made from sheep and goats skins (Watt and Breyer-Brandwijk, 1932; Moffett, 2010; Moteetee and Van Wyk, 2011).

According to Okello and Ssegagwa (2007), in Uganda, the pounded root extract of *R. densiflora* is given to cattle in a dose of 2–3 times a day for the treatment of dysentery. Furthermore, in Ethiopia, the roots are used to treat diarrhoea and also as an antiemetic (Lulekal et al., 2008).

Rhynchosia harveyi is the least utilised plant species of *Rhynchosia* in southern Africa as it is only recorded to be of medicinal use in the Eastern Cape Province in South Africa.

This is not surprising since its natural distribution is confined to the Eastern Cape. The pulverised root powder is sniffed to relieve headaches (Neuwinger, 2000).

Rhynchosia hirta is mostly used in East Africa, for treating a number of ailments. The plant is used to obtain a speedy and painless delivery and for the removal of the afterbirth placenta. The root is used for the treatment of children's disease known as "*hudha*", which is bilharzia (schistomiasis) in the local '*Luo*' language, and also as a purgative (Kokwaro, 1976; Neuwinger, 2000). A mixture of roots and leaves is utilised for stomach problems, and in cases of kwashiorkor a leaf decoction is administered as an enema (Kokwaro, 1976; Neuwinger, 2000). Jeruto et al. (2010, 2011) reveal that the root decoction of *R. hirta* can be a good remedy for stomach ulcers, cancers, and also in the treatment of malaria in Nandi district of Kenya. In India, only the leaves are used to treat stomach disorders (Amjad et al., 2015). The leaf-extract of *R. hirta* is a good cure for otitis, leaf extract or leaf sap drops are administered into the ear of the patient, while the whole plant is also used externally to treat elephantiasis (Neuwinger, 2000).

Rhynchosia minima (least snout bean) has emerged to be the most commonly used plant species among the nine medicinally important *Rhynchosia* species. This is not surprising as it is also the most widespread occurring in southern Africa throughout tropical Africa, extending as far as India and China in the east, it also occurs in Cape Verde Islands, tropical America and the Middle East countries including Israel and Afghanistan (Verdcourt, 2001). According to the literature, both roots and leaves are used in African countries, whereas in Asian countries leaves are mostly used for the treatment of various diseases, including gastro-intestinal illnesses caused by pathogens associated with diarrhoea (Neuwinger, 2000; Chaudhary, 2010; Maheswari et al., 2012; Singh et al.,

2013; Yellasubbaiah et al., 2015; Kshirsagar, 2016); respiratory problems such as tuberculosis and asthma (Neuwinger, 2000; Chaudhary, 2010; Singh et al., 2013; Jia et al., 2015), skin conditions (e.g. healing of wounds and alleviation of boils) (Gundidza et al., 2009; Maheswari et al., 2012; Singh et al., 2013; Jia et al., 2015; Yellasubbaiah et al., 2015; Kumudha and Yogeshwari, 2016; Phondani et al., 2016), urinary bladder and kidney infections, bilharzia (Schistomiasis) (Neuwinger, 2000), and STIs such as gonorrhoea, wherein the root decoction is used (Chhabra and Mahunnah, 1994; Neuwinger, 2000). It is also used for various ailments such cardiac pains, chronic fever, joint pains, and menopausal problems (Neuwinger, 2000; Jia et al., 2009; Kanthale and Biradar, 2012). In India, the leaves of *R. minima* are used as an abortifacient (Chopra et al., 1956; Mali et al., 2006; Jain et al., 2009; Chaudhary, 2010; Rothe, 2011; Kumar et al., 2012; Singh et al., 2013; Rekha et al., 2014; Johnson et al., 2015; Yellasubbaiah et al., 2015; Basha and Reddy, 2017). The seeds of the least snout bean are also used as tonic and antibiotic (Neuwinger, 2000; Singh, 2006; Rothe, 2011; Jain et al., 2015). In Tanzania, *R. minima* is mixed with other plants (*Cassia gracilior* (Ghesq.) Steyaert, *Antherotoma naudinii* Hook.f., *Dissotis brazzae* Cogn., *Indigofera asparagoides* Taub., *Justicia diclipteroides* Lindau, and *Pentas zanzibarica* Vatke) and drunk for the treatment of malaria (Chhabra and Mahunnah, 1994).

Rhynchosia resinosa is commonly used for the treatment of gastro-intestinal illness associated with diarrhoea and ulcers, mostly root infusion is used (Gelfand et al., 1985; Anokbonggo et al., 1990; Neuwinger, 2000). In India, leaf decoction is mixed with other species (*Ozoroa insignis* Delile, *Gymnosporia senegalensis* (Lam.) Loes, *Entada abyssinica* Steud. ex A. Rich. and *Lannea schimperi* (Hochst.ex A. Rich.) Engl.) and used

as a traditional cure for peptic ulcers (Barik et al., 2014). It is also utilised in infertility (in Malawi, branches infusion is used for making thick a porridge, then eaten), for the relieve of abdominal pains where the root infusion is taken orally to induce labour in pregnant women, where the root powder is inserted into the vagina, to dilate the birth canal and also for painful menstruation (dysmenorrhoea) in which the root infusion is drunk (Gelfand et al., 1985; Neuwinger, 2000). According to Ssegagwa and Kasenene (2007), in Uganda a leaf decoction is drunk to treat STIs such as syphilis. *Rhynchosia resinosa* is also used in the promotion of wound healing, the wound is washed with an infusion from the whole plant to expel maggots (Gelfand et al., 1985; Neuwinger, 2000).

Rhynchosia sublobata is the second most widely distributed and used after *R. minima*. It occurs naturally in the wild in Namibia, Botswana, northeast South Africa into eSwatini, Mozambique and Zimbabwe, northwards to broader region of Africa, including Central African Republic, Democratic Republic of Congo, Sudan, Somalia, East Africa, West Africa and Yemen (Verdcourt, 2001). It is also found in Madagascar and Comores Islands. The root (sometimes leaves are used) infusion or decoction is used for the treatment of stomach disorders, including diarrhoea (also bloody diarrhoea) and dysentery (Kokwaro, 1976; Neuwinger, 2000; Maregesi et al., 2008; Bruschi et al., 2011). In Mozambique, the root infusion is used for the treatment of stomach aches and backache of the mother during pregnancy (Bruschi et al., 2011). In Tanzania and Kenya, root decoction and leaf sap are used in the treatment of tachycardia, gall bladder disorders in children, and chest illnesses (Kokwaro, 1976; Neuwinger, 2000; Maregesi et al., 2008). Root decoction is drunk to treat urinary incontinence and dizziness, and also used as a mouth wash to cure tooth diseases, while the root infusion is drunk (hot) as a

remedy for pneumonia, and the pulverised root is sniffed to relieve headaches (Neuwinger, 2000). The leaf pulp is used as dressing in swollen knees and for the treatment of articular rheumatism (Neuwinger, 2000). According to Neuwinger (2000), it is also used to cure scabies, where it is mixed with palm oil and rubbed on the skin. The roots and bark are also used as antidote for snake bites (Kokwaro, 1976; Maregesi et al., 2008).

It is quite intriguing to note that although these species naturally occur in the wild in southern Africa, only a few ethnobotanical surveys carried out in the region have listed a few records of *Rhynchosia* species known to be used medicinally, for example in a study by Shale et al. (1999) only *R. adenodes* is listed. In his book on plants used by the Basotho, Moffett (2010) listed only two species (*R. adenodes* and *R. caribaea*) as medicinally important. Recently, *Rhynchosia villosa* was added from the Eastern Cape, used by the Xhosa people to treat skin conditions such as swollen body organs, and also to enhance the skin texture and tone (Rondo, 2017). Although several ethnobotanical surveys have been carried out in different parts in South Africa recently (De Wet et al., 2010; Omoruyi et al., 2012; Semenya and Maroyi, 2012; Semenya et al., 2012; Mahwasane et al., 2013; Semenya et al., 2013; Afolayan et al., 2014; Hughes et al., 2015), no *Rhynchosia* species, including the widespread *R. minima*, have been recorded to be used medicinally.

Although the ethnobotanical scope was limited to published literature in this study, the results do provide a basis for further study, where more information can be gathered through questionnaires to unearth more indigenous knowledge of ethnobotanical uses of *Rhynchosia* species.

4.3.2. Antimicrobial screening

The results of the antimicrobial activity of extracts from three species (*R. adenodes*, *R. hirta* and *R. sublobata*) are presented in Table 4.4. All aqueous extracts showed poor activity against all bacteria tested, with an MIC value of above 8. 00 mg/ml. This is not suprising as aqueous plant extracts generally yield a low activity (Pauw and Eloff, 2014). Traditional health practitioners use water to prepare their medicines, but it has been suggested that perhaps the effective use of these method is based on an indirect effect catylising the host's immune system than effectively killing the pathoges (Pauw and Eloff, 2014). On the other hand, organic extracts showed better activity, with the highest activity (0.125 mg/ml) exhibited by root extracts of *R. sublobata* against *E. coli* (a Gram-negative bacterium), followed by a moderate activity (0.625 mg/ml) of *R. hirta* and *R. sublobata* against *S. aureus* (a Gram-positive bacterium) and *R. hirta* against *E. coli. Rhynchosia adenodes* extracts displayed the poorest activity, with the highest activity at 0.875 mg/ml against *S. aureus* and at 1.750 mg/ml against *E. coli.* The remaining pathogens, i.e. *Enterococcus faecalis, Proteus vulgaris,* and *Pseudomonas aeruginosa* were resistant to all the extracts. The latter two are both Gram-negative bacteria, known to be resistant due to their outer membrane that is made of thin lipopolysaccharide and is impermeable to most antimicrobial compounds, versus Gram-positive bacteria with a permeable mesh-like peptidoglycan layer (Madikizela et al., 2012; Biswas et al., 2013). It is therefore not surprising that none of the extracts inhibited their growth. Gram-negative bacteria's resistance is a serious challenge to treatment of human infections (Abdou et al., 2011). The poor living conditions of people, especially rural communities contribute to much suffering from diarrhoea and vomiting, as a result the people resort to indigenous plants

as remedies. In many cases, diarhoea is a result of contaminated food caused by pathogens such as *Salmonella typhi*, *Campylobacter jejuni* and *Escherichia coli*, and the drinking of contaminated water infested with *Giardia intestinalis* and *Cryptosporidium parvum* (Mathabe et al., 2006; Madikizela et al., 2012). In this regard, the efficacy of *R. hirta* and *R. sublobata* in the treatment of stomach disorders and respiratory problems, appears to be supported, however, further investigation needs to be done to acertain the findings of this study.

Table 4.4. Antimicrobial activities of plants used against gastro-intestinal, urinary tract infections, respiratory and skin conditions (values in bold indicate noteworthy activity; values in italics indicate moderate activity).

Plant Species	**Voucher**	**Parts used**	**Antimicrobial activity (MIC values in mg/ml**									
			Ef (ATCC29212)		**Ec (ATCC8739)**		**Pv ATCC33420)**		**Pa (ATCC27853)**		**Sa (ATT25923)**	
			Organic	**Aqueous**	**Organic**	**Aqueous**	**Organic**	**Aqueous**	**Organic**	**Aqueous**	**Organic**	**Aqueous**
Rhynchosia adenodes	MSM & TTM 06 (PRE)	Roots	4.000	>8.000	1.750	8.000	2.000	4.000	4.000	>8.000	0.875	8.000
Rhynchosia hirta	MSM 891 (JRHAU; PRE)	Leaves	4.000	>8.000	3.000	>8.000	4.000	>8.000	4.000	>8.000	4.000	>8.000
		Roots	4.000	>8.000	*0.625*	>8.000	4.000	>8.000	4.000	>8.00	*0.625*	>8.000
R.sublobata	MSM 890 (JRHAU; PRE)	Leaves	4.000	>8.000	6.000	>8.000	4.000	>8.000	4.000	>8.00	4.000	>8.000
		Roots	4.000	>8.000	**0.125**	>8.000	2.000	>8.000	4.000	>8.00	0.500	>8.000
Ciprofloxacin		+ C	0.039	0.039	0.078	0.078	0.020	0.020	0.313	0.313	0.078	0.078
Acetone		-C	>8.000	>8.000	>8.000	>8.000	>8.000	>8.000	>8.000	>8.000	>8.000	>8.000

Pathogens: *Ef, Enterococcus faecalis; Ec, Escherichia coli; Pv, Proteus vulgaris; Pa, Pseudomonas aeruginosa; Sa, Staphylococcus aureus*. MIC, Minimum Inhibitory Concentration with values in mg/ml. +C, positive control; -C, negative control.

4.3.3. Phytochemistry

The current study provides qualitative phytochemical evaluation of selected *Rhynchosia* species (Table 4.5). The results of qualitative phytochemical screening indicated that alkaloids were detected in all six *Rhynchosia* species studied (*R. adenodes*, *R. albissima*, *R. caribaea*, *R. hirta*, *R. minima* and *R. sublobata*), and they were mostly detected in the leaf extracts of the plants than in the roots. Alkaloids were detected in all tested species's water extracts, except for *R. hirta* where no detection was made. Alkaloids were better detected in methanol as compared to extracts of acetone and water. Flavonoids were detected in all six tested species. Water as a polar molecule, was found to be an excellent solvent to extract flavonoids, better than acetone or methanol. Their presence was found to be positive in both leaves and roots. *Rhynchosia hirta* emerged as the only species that lacked terpenoids. Terpenoids were poorly detected in both water and acetone extracts, and methanol was found to be a good solvent for extraction of terpenoids. Terpenoids were found to be present in roots of *R. adenodes*, *R. caribaea* and *R. minima*, except in *R. albissima* and *R. sublobata* where their presence was detected in leaves and roots.

The foam test was performed to detect the presence of saponins. Saponins were positively detected in water extracts of all species, with the exception of *R. adenodes* were only detected in methanol extract. Saponins were found to be present in root extracts of the studied species, with the exception of *R. albissima* and *R. sublobata* where their presence was detected in both leaves and roots extracts.Water showed to be a poor extraction solvent for tannins. However, tannins were positively detected in all species and in both leaves and roots in the acetone and methanol extracts. Coumarins were not

detected in five of the six investigated species (*R. adenodes*, *R. albissima*, *R. hirta*, *R. minima* and *R. sublobata*), except in leaves and roots of *R. caribaea*. Phenols were detected in all studied plant species and in all water extracts, acetone and methanol. They were also found to be present in leaves and roots. Phenols have been used in the treatment of inflammation, fever and chronic pain, and have an antioxidant activity (Kähkönen et al., 1999; Van Wyk and Wink, 2015).

Rhynchosia adenodes and *R. caribaea* exhibited the presence of flavonoids, terpenoids tannins and phenols. In addition, *R. caribaea* has also shown the presence of coumarins. Root decoctions of these plants are used for the treatment of rheumatic pains in Lesotho (Moteetee and Van Wyk, 2011). Neuwinger (2000) defines '*rheumatism'* as a disease marked by inflammation and pain in the muscles, joints or fibrous tissue of the body. Over centuries flavonoids have been promoted as anti-inflammatory agents (Bruneton, 1999), they have been found to possess pharmacological activities including antioxidant, hepatoprotective, antibacterial, anti-inflammatory, anti-cancer, anti-viral, anti-ulcer, anti-hyperlipidemia, and analgesic activities (Kumar and Pandey, 2013; Yin et al., 2015). Terpenoids exhibit numerous important pharmacological activities including anti-inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and ant-bacterial activities (Wadood et al., 2013). Tannins are strong antioxidants, with anti-inflammatory, antibacterial, antifungal and antiviral activities (Van Wyk and Wink, 2015; Coumarins are used in phytomedicine due to their anti-inflammatory and antimicrobial properties (Van Wyk and Wink, 2015). Therefore, the efficacy of *R. adenodes* and *R. caribaea* species for the management of rheumatism might be explained by the chemical composition of this plant, including anti-inflammatory properties. *Rhynchosia adenodes*

is also used for the treatment of dysentery in calves. Dysentery is defined as an inflammation of the colon, often caused by bacteria or viruses, accompanied by pain and severe diarrhoea (Van Wyk and Wink, 2015). This often results in ulceration of the bowel and occasionally in the formation of abscesses in the liver, lungs, testes or brain (Neuwinger, 2000). The efficacy of this species could be due to the presence of flavonoids, terpernoids, tannins and phenols, with pharmacologiacal properties such as anti-ulcer, anti-inflammatory, antibacterial and analgesic activities.

Rhynchosia albissima displayed the presence of alkaloids and tannins. The plant is used to induce delivery (when mixed with *Tetracera parviflora*) in Tanzania and India (Hedberg et al., 1983; Lima et al., 2014). Pharmacological properties of alkaloids include analgesic, central nervous stimulant, central nervous depressant, antihypertensive, antihypertensive, antipyretic, anticholinergic, anti-emetic, oxytocic and vaso constrictor, antitumor and antimalarial activities (Cushnie et al., 2014). Tannins are also used traditionally to halt diarrhoea and as vasoconstrictors (Van Wyk and Wink, 2015). The efficacy of this species in the inducement of labour in delivery could be due to the presence of these chemicals. Tannins were detected in the roots better than in the leaves of *R. albissima*.

Rhynchosia hirta has exhibited positive detection of alkaloids, flavonoids and phenols. It is recorded that the root decoction of *R. hirta* is used for the treatment of the above mentioned illnesses. The successful use of *Rhynchosia hirta* in the treatment of these disseases is possibly due to the presence of these chemicals (flavonoids, tannins and phenols).

In *Rhynchosia minima,* the presence of alkaloids, flavonoids, terpenoids and tannins were detected. *Rhynchosia minima* is used to treat various ailments including stomach problems (diarrhoea, peptic ulcer, etc.). Terpenoids have been found to have anti-HIV, antitumor, anti-aging, immunosuppressant, antiprotozoal and antihelminth activities (Vaishnav and Demain, 2010). The efficacy of this plant in the management of stomach disorder is perhaps because of the pharmacological properties of these chemical compounds in the plant. The antipyretic property of alkaloids reduces fever and together with terpenoids, tannins and phenols, helps with alleviation of inflammation and bacterial infection in the respiratory system, therefore the use of *R. minima* in respiratory infections might be scientifically reasonable. Sexually transmitted infections (STI's) are caused by viral infections and fungi. The presence of anti-viral and anti-HIV (terpenoids) properties and anti-fungal (tannins) properties, perhaps explains the efficacy of the species. Phenols are good in the treatment of inflammation, fever and chronic pain (Van Wyk and Wink, 2015).

Alkaloids, flavonoids, terpenoids, saponins and tannins were positively detected in *Rhynchosia sublobata*. The efficacy of this species in the treatment of stomach problems can be explained by presence of these compounds. Tachycardia is an excessive rapidity in the action of the heart (heart rate of above 100 beats per minute in an adult) (Neuwinger, 2000). The pharmacological properties of this plant are believed to be responsible for the efficacy of this plant in the management of heart problems. In addition, *R. sublobata* contains saponins. Saponins are the glycosides of triterpenes or steroids and include the group of cardiac glycosides and steroidal alkaloids (Van Wyk and Wink, 2015). These type of saponins slow down the heart beat and exhibit positive inotropic,

positive bathmotropic, weakly negative chronotropic and dromotropic heart activity (Van Wyk and Wink, 2015).

Human illnesses including bladder infections, scabies, chest illnesses and pneumonia, are often associated with bacterial infections, accompanied by fever in the case of pneumonia and other chest illnesses. Therefore, the successful alleviation or management of these illnesses using the above plant species appears to be due to the presence of these chemical compounds.

Table 4.5: Phytochemical screening of leaves and roots extracts of selected *Rhynchosia* species used for various ailments.

Species	Vouchers	Parts used	Alkaloids			Flavonoids			Terpenoids			Saponins			Tannins			Coumarins	Phenols		
			H_2O	AcOH	MeOH	H_2O	AcOH	MeOH	H_2O	AcOH	MeOH	H_2O	AcOH	MeOH	H_2O	AcOH	MeOH	EtOH	H_2O	AcOH	MeOH
Rhynchosia adenodes	MSM & TTM 06 (PRE)	Lvs	-	+	+	+	+	+	-	-	-				+	+	+	-	+	+	+
		Rts	+	-	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	+	+
Rhynchosia albissima	MSM & TTM 20 (PRE)	Lvs	+	-	+	+	-	+	+	-	-	+	-	-	+	+	+	-	+	+	+
		Rts	-	+	+	+	-	+	+	-	+	+	-	-	+	+	+	-	+	+	+
Rhynchosia caribaea	MSM & KWS 12 (PRE)	Lvs	+	+	-	+	-	+	-	-	-	-	-	-	-	+	+	+	+	+	+
		Rts	-	-	-	+	+	+	-	+	+	+	-	-	-	+	+	+	+	+	+
Rhynchosia hirta	MSM 891 (JRHAU; PRE)	Lvs	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	+	+	+
		Rts	-	+	+	+	-	-	-	-	-	+	-	-	-	+	+	-	+	+	+
Rhynchosia minima	MSM 850B (PRE)	Lvs	+	+	-	+	+	+	-	-	-	-	-	-	-	+	+	-	+	+	+
		Rts	-	-	+	+	+	+	-	-	+	+	-	-	-	+	+	-	+	+	+

Rhynchosia sublobata	MSM 890 (JRHAU; PRE)	Lvs	+	-	+	-	+	-	-	-	+	+	-	+	+	+	+	-	+	+	+
		Rts	+	-	+	+	-	-	-	-	+	+	-	-	+	+	+	-	+	+	+

Solvents: H_2O-Water, AcOH-Acetone, MeOH-Methanol, EtOH-Ethanol. Symbols: + positive test, - negative test. Plant parts: Lvs-Leaves, Rts-Roots.

CHAPTER 5: GENERAL CONCLUSIONS AND FUTURE RESEARCH

5.1 General conclusion

The primary aim of this study was to resolve the taxonomic uncertainties in the *Rhynchosia minima* complex species, which are medicinally important within the type section. The study also set out to evaluate the ethnomedicinal uses, assess the antimicrobial actitities, and conduct a qualitative screening of the phytochemical compounds of these species. The findings are summarized as follows:

5.1.1. Taxonomic revision of *Rhynchosia minima* complex

The findings of this treatment, present five varieties of *R. minima* complex. The varieties of this complex are characterized by erect and prostrate herbs, mostly with long twinning stems arising from woody rootstock, and bluish-green or yellowish green, gland dotted leaflets of variable sizes. They are widely distributed across five countries in southern Africa (Botswana, Namibia, South Africa, Lesotho and eSwatini). Two new varieties from southern Africa (*R. minima* var. *glandularis* and *R. minima* var. *magniflora*) are described. *Rhynchosia minima* var. *glandularis* is a prostrate herb with long trailing stems. Its leaflets are yellowish-green, glabrescent or with fine pubescence and have glands distributed on both sides. It grows on red sandy soils, among rocks and along roadsides and is restricted to Namibia. *Rhynchosia minima* var. *magniflora* is a prostrate herb with grey-green leaflets, covered with fine pubescence and dotted with golden glands in the lower surface. It has distinctly larger flowers (9–12 mm long) than all the other varieties. *Rhynchosia minima* var. *magniflora* occurs in the grasslands of South African provinces (Limpopo,

Gauteng, Mpumalanga and KwaZulu-Natal) and in eSwatini. *Rhynchosia minima* var. *falcata* is sunk into *R. minima* var. *minima*.

5.1.2. Synopsis of selected medicinally important *Rhynchosia* species in southern Africa

Rhynchosia adenodes can easily be recognized by its ovate-orbicular leaves, glabrescent, with tiny red glands on the lower surface and a much longer petiole (5–35 mm). The species occurs in South Africa and Lesotho, with its geographic distribution from the Western Cape in the South, through Eastern Cape and KwaZulu-Natal into Lesotho, and northwards to Limpopo Province.

Rhynchosia albissima is a robust woody shrub reaching up to 3m high. The stem is rigid, the leaves covered with dense silvery-grey hairs. It is found in grasslands in northeast part of South Africa (Limpopo, North West, Gauteng, Mpumalanga and KwaZulu-Natal) eastwards to eSwatini and Mozambique, and northwards to Somalia in East Africa.

Rhynchosia caribaea is a herbaceous climber, characterized by heart-shaped leaflets that are glabrescent or have fine pubescence, covered in glands. The fruits are sub-inflated, covered with sparse to dense long silky-glandular hairs. It is found in grasslands, near streams, and forest margins in Namibia, Botswana, all provinces of South Africa, except Northern Cape, and eSwatini.

Rhynchosia harveyi is a prostrate or climbing herb with long stems trailing along the ground. The leaflets are small, covered with fine hairs, and with short petioles of 5–15 mm long. It is restricted to the Eastern Cape Province of South Africa.

Rhynchosia hirta is an ever-green climber, with broadly eliptic-ovate leaflets, which are characteristically large. Flowers large (10–25 mm long), with variable bright colours of bluish-grey, mauve or yellow. The seed is distinctly brown or dark bluish-black. This species is found on the sandy grasslands, outcrops, loam soils of open woodlands and the margins of moist forest in Limpopo and Mpumalanga Provinces.

Rhynchosia resinosa is an erect woody climbing shrub, up 3 m long, often aromatic, with sticky stems. The leaves are broadly rhombic, characteristically triangular and paler beneath. The fruits are straw coloured, with dark reddish-brown, marked with black spots seeds. It is found naturally in the river forest marguns, rocky areas of the the northeastern part of South Africa (Limpopo Province), Namibia, Botswana, Mozambique and Zimbabwe northwards into tropical Africa countries.

Rhynchosia sublobata is a woody prostrate climbing herb, with rhombic-ovate leaves, covered with hairs. The flowers are yellow, cream, stripped with red or brown or purple marks. The fruit is distinctly inflated, with no or minute pubescence. It has a wide distribution, spreading from Namibia, Botswana, northeast of South Africa (Limpopo and Mpumalanga Provinces) into eSwatini, Mozambique and Zimbabwe, northwards to broader region of Africa.

5.1.3. Ethnobotany, antibacterial activity and phytochemistry of medicinal species of *Rhynchosia*

5.1.3.1. Ethnobotany

The literature survey has revealed that there are ten *Rhynchosia* species occurring in southern Africa that are medicinally used for the treatment of various diseases. The

species are: *Rhynchosia adenodes*, *R. albissima*, *R. caribaea*, *R. densiflora*, *R. harveyi*, *R. minima, R. resinosa, R. sublobata* and *R. villosa*. The use of these plants is not limited to the region. In this region only four species are used (i.e. *R. adenodes*, *R. caribaea*, *R. harveyi* and *R. villosa*) while the other six are used in the Asian countries such as China and India, as well as in East Africa (Kenya, Tanzania, Uganda, etc.). Six species are used for the treatment of gastrointestinal disorders (e.g. diarrhoea, etc.), two species for the treatment of RTIs such as coughs, colds and asthma; four species for skin conditions such as boils and promotions of wound healing, and two species for STIs including gonorrhoea, syphilis, etc. The species are also used as aphrodisiacs and for the treatment of rheumatic pains, male impotence, menstrual pains, headaches, elephantiasis, bilharzia, otitis, as snake bite antidote, expulsion of the retained placenta and in the inducement of quick and painless delivery. These findings will contribute to the ethnobotanical knowledge on the traditional uses of *Rhynchosia* species in South Africa and the region.

5.3.1.2. Antimicrobial activity

To the best of our knowledge, none of the *Rhynchosia* species tested in this study, except for *R. minima*, have previously been evaluated. Due to the fact that *R. minima* has been extensively assessed for antimicrobial activity, only three species (*R. adenodes, R. hirta* and *R*. *sublobata*) were evaluated in the current study. The extracts were tested for their efficacy against some of the common causative pathogens prevalent in respiratory and gastrointestinal infections, namely; *Enterococcus faecalis*, *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus*. All aqueous extracts

exhibited low activity against all tested pathogens, while on the other hand the organic extracts exhibiting a moderate to noteworthy activity, especially the root extracts of *R. hirta* against *E. coli* and *R. sublobata* against *S. aureus*, bacterium. Interestingly, in a previous study by Gundidza et al. (2009), *R. minima* extracts showed some antibacterial activity against *E. coli*, *P. vulgaris*, *P. aeruginosa* and *S. aureus*, whereas in the current study, *Pseudomonas aeruginosa* and *Proteus vulgaris* proved to be resistant to all the tested *Rhynchosia* species. Furthermore, a study by Challa et al. (2011) showed that *R. scarabaeoides* (L.) DC. extracts exhibited high activity against *S. aureus* and *P. vulgaris*. Our findings suggest that extracts of *R. hirta* and *R. sublobata* have potential as antibacterial agents, and could be useful for further pharmacological studies, including their toxicity levels. These findings contribute new knowledge with regards to antimicrobial studies of *Rhynchosia* species.

5.3.1.3. Phytochemistry

The findings of the qualitative phytochemical screening indicated the presence of various chemical compounds including alkaloids, coumarins, flavonoids, phenols, tannins, terpenoids and saponins. The total of six species were studied (*R. adenodes*, *R. albissima*, *R. caribaea*, *R. hirta*, *R. minima* and *R. sublobata*), and to the best of our knowledge the phytochemistry of the following species: *R. adenodes, R. albissima, R. caribaea, R. hirta* and *R. sublobata*, have not previously been tested. Alkaloids, flavonoids, phenols and tannins were found in all six species; terpenoids were detected in all, except in *R. hirta*. Coumarins were only found in *R. caribaea.* Previous phytochemical studies showed that *R. minima* and *R. beddomei* possess similar chemical

coumpounds in addition to essential oils, carotenoids, steroids and amino acids. The findings of this study, present new knowledge on the chemistry of *Rhynchosia* species.

5.2. Future Research

- This taxonomic study focussed on the *R. minima* complex, and the species that are ethnomedicinally useful within the type section (*Rhynchosia*). The current work in *Rhynchosia* is so far been based on sectional or regional level, therefore future work in southern Africa should be dedicated to a revision of the genus as a whole.
- Due to the scope of this study, ethnobotanical information based on field surveys involving questionnaire surveys could not be sourced. It is recommended that future studies should include such surveys to increase the possibility of more plants being discovered.
- The findings of this study on the antimicrobial activity of the *Rhynchosia* species serve as a basis for further investigation, especially for the other ethnomedicinal useful species such as *R. densiflora* and *R. resinosa,* which could not be found in the field.
- The work in this study demonstrated that the phytochemistry of *Rhynchosia* is understudied, therefore further investigation need to be undertaken especially for all the ethnomedicinallly important species.

CHAPTER 6: REFERENCES

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